Intergovernmental Oceanographic Commission
Training Course Reports

19

IOC-SOA Training Workshop on Environmental Effects on Benthic Communities

Xiamen, China, 19-23 October 1992

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IOC Training Course Reports

No.	Title	Language versions
1.	IOC Indian Ocean Region Training Course in Petroleum Monitoring Perth, 18 February-1 March 1980	English
2	IOC Regional Training Course for Marine Science, Technicians Cape Ferguson, Queensland, 1-28 June 1980	English
3.	ROPME-IOC-UNEP Training Workshop on Oceanographic Sampling Analysis, Data handling and Care of Equipment, Doha, Qatar, 3-15 December 1983	English
4.	Stage COI d'initiation à la gestion et au traitement de l'information scientifique et technique pour l'océanologie, Brest, France, 28 novembre - 9 décembre 1983	French
5	Curso mixto COI-OMM de formación sobre el Sistema Global Integrado de Servicios Oceánicos (SGISO), Buenos Aires, Argentina, 15-26 de octubre de 1984	Spanish
6	UNESCO-IOC-NBO Training Course on Tidal Observations and Data Processing Tianjin, China, 27 August - 22 September 1984	English
7.	Stage COI sur la connaissance et la gestion de la zone côtière et du proche plateau continental Valence, France, 18 septembre - 4 octobre 1984	French
8	IOC Regional Training Course on Marine Living Resources in the Western Indian Ocean Mombasa, Kenya, 27 August - 22 September 1984	English
9.	IOC-UNESCO Summer School on Oceanographic Data, Collection and Management Erdemli, Icel, Turkey, 21 September - 3 October 1987	English
10.	IOC-UNESCO Regional Training Workshop on Ocean Engineering and its Interface with Ocean Sciences in the Indian Ocean Region, Madras, India, 17 March - 5 April 1986	English
11.	IOC-UNESCO Training Course on the Use of Microcomputers for Oceanographic Data Management Bangkok, Thailand. 165 January - 3 February 1989	English
12.	IOC Advanced Training Course on Continental Shelf Structures Sediments and Mineral Resources Quezon City, Philippines, 2-13 October 1989	English
13.	IOC/IODE Training Course on GF3 Data Formatting System Obninsk, USSR, 14-24 May 1990	English
14.	IOC Training Course on Microcomputers and Management of Marine Data in Oceanographic Data Centres of Spanish-speaking Countries, Bogotá, Colombia, 21-30 October 1991	English Spanish
15.	IOC Advanced Training Course on Nearshore Sedimentation and the Evolution of Coastal Environments, Kuala Lumpur, Malaysia, 17-29 February 1992	English
16.	First IOC Training Course on the Applications of Satellite Remote Sensing to Marine Studies Caracas, Venezuela, 24-28 September 1990	English
17.	IOC-KMFRI-RECOSCIX (WIO) Regional Training Course on Microcomputer-based Marine Library Information Management, Mombasa, Kenya, 10-21 August 1992	English
18.	ROPME-IOC Regional Training Course on Management of Marine Data and Information on Microcomputers for the ROPME Region, Kuwait, 18-28 October 1992	English
19.	IOC-SOA Training Workshop on Environmental Effects on Benthic Communities Xiamen, China, 19-23 October 1992	English

TABLE OF CONTENTS

1. INTRODUCTION

The first IOC research workshop on the Biological Effects of Pollutants, organized by the Group of Experts on the Effects of Pollutants (GEEP) under the auspices of the IOC Global Investigation of Pollution in the Marine Environment (GIPME) programme, took place in Oslo, Norway, 8-30 August, 1986. Thirty-two scientists from eleven countries undertook practical testing of a range of methods for detecting biological consequences of contaminants in the marine environment, by collecting and analyzing data from a field pollution gradient and in mesocosm experiments. Particularly well-represented were techniques for analyzing community changes, to macrobenthic and meiobenthic assemblages. This workshop and associated research have shown the sensitivity and reliability of newer methods of statistical (graphical and multivariate) analysis in detecting and interpreting pollution effects, on soft-sediment communities in particular.

This, and the full range of successful methods based on individual organism physiology, cellular and biochemical responses etc., are reported in detail in the IOC Workshop Report no. 53 and a Special Issue of Marine Ecology Progress Series (1988, volume 46).

The second IOC/GEEP research workshop, Bermuda, 10 September-2 October 1988, then followed this up by investigating the validity of some of these techniques to sub-tropical organisms and communities. On the benthic community side, this demonstrated the feasibility of using data of a relatively coarse level of taxonomic discrimination (and thus cost-effectively collected) to demonstrate rather subtle changes in community structure. This workshop was reported in IOC Workshop Report no. 61 and in a Special Issue of the Journal of Experimental Marine Biology and Ecology (1990, volume 138). A further research workshop, jointly sponsored by IOC and ICES, examined "biological effects" techniques in the context of shelf rather than coastal studies, with the constraints this imposes on sampling methods (North Sea, 1990).

This series of research workshops spawned a number of FAO/UNEP/IOC training activities, especially in the area of training in newer methods of statistical analysis and interpretation of community data, within the framework of the long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL - Phase II). The present workshop, however, was the first attempt to draw together participants from outside the Mediterranean, for a regional IOC training workshop on methods of data analysis, with particular reference to benthic community studies within the WESTPAC region.

This IOC Training Workshop took place from 19-23 October 1992 at the Third Institute of Oceanography, State Oceanic Administration (SOA), Xiamen, Fujian, China. It was attended by 15 participants, 8 from a wide range of locations within China (Beijing, Dalian, Qingdao, Shanghai, Hangzhou, Xiamen), and 7 from various countries in the WESTPAC region (Thailand, R.O. Korea, D.P. R. Korea, Vietnam, Malaysia, Indonesia). The list of participants is given in Annex I.

The lectures on methodology and interpretation were given by Drs Robert Clarke and Richard Warwick (Plymouth Marine Laboratory, UK), and on software by Dr Martin Carr (PML). Mr Yihang Jiang attended on behalf of IOC, and a local organizing committee under Prof Qiulin Zhou, with principal co-ordinator Dr Yusheng Zhang, were responsible for the arrangements within Xiamen.

2. SCIENTIFIC CONTENT

The workshop covered the statistical treatment of community data (species abundances/biomass), arising in studies of the marine environment. In particular, the emphasis was on statistical analysis of the biological effects of pollutants, the workshop being conducted through lectures and practical computing sessions involving a range of data sets drawn from the literature, particularly research on responses of benthic communities.

The methods covered ranged from "classical" diversity indices, to multivariate clustering and ordination techniques, and other graphical methods, applied to large arrays of samples/species data. Practical work on multivariate analyses was undertaken using the package PRIMER (Plymouth Routines In Multivariate Ecological Research), a suite of PC programmes written at the Plymouth Marine Laboratory, UK.

The lecture material covered:

a) the use of multivariate methods (clustering and ordination) to represent graphically the similarities between species abundances (or biomass) observed in a set of samples;

b) the demonstration of statistically significant differences in species composition between several sites (or the same site at several times) - this is a necessary pre-requisite to further analyses attempting to explain those differences;

c) the construction of univariate indices (e.g. diversity) and distributional plots (e.g. abundance-biomass comparisons) which indicate levels of disturbance or "stress" at sites;

d) the relation of both univariate and multivariate faunal descriptions to gradients of chemical contamination and background environmental variables.

The practical sessions allowed the participants to apply the methods described in the accompanying lectures, on published data sets chosen to illustrate changes in benthic community composition at macrofaunal and meiofaunal levels, resulting from contaminant impact by sewage sludge dumping, pulp mill effluent, oil spills etc.

3. PROGRAMME OF THE WORKSHOP

The Workshop took place over 5 days of intensive lecturing, practical computing exercises and discussion sessions. It commenced with a formal ceremony opened by the Director of Planning, Science and Technology of the Third Institute at Xiamen, Prof Quilin Zhou, who gave a detailed and entertaining introduction to the history of the Third Institute, its current role and past and present links with laboratories worldwide. On behalf of the Third Institute, its Deputy Director, Dr Ziqiang Huang, formally welcomed the lecturing team from Plymouth, the IOC organizer and the participants from China and abroad, and went on to point out the importance of benthic community monitoring to marine environmental protection and the benefits of access to advanced statistical techniques to studies within the Third Institute and in developing countries in the Western Pacific and Asia. He also invited participants to tour the laboratory later in the week and welcomed everyone to the district of Xiamen, a beautiful island city enjoying clement weather all the year round.

From the Department of Science and Technology of the SOA, its Deputy Director, Dr Peizhong Wu, added his welcome and best wishes for the success of the workshop, then turned his attention to the continuous and growing effects of human activities on the world's marine environment and resources. He applauded the activities undertaken by various UN organizations, in particular the IOC, on the issues of marine pollution, and pointed also to the efforts of the Chinese government and scientists. At that very moment, the Third Institute was also hosting another international Training Course, in Development and Management of the Exclusive Economic Zone, and its "macroscopic" approach to marine environmental problems was a perfect complement to the current workshop's "microscopic" approach.

On behalf of the IOC, Mr Yihang Jiang then expressed his thanks for all the efforts and co-operation of the Third Institute in organizing and providing necessary facilities to the Workshop, to the lecturers for preparing and delivering timely the training material 4 months before the Workshop, and particularly to the SOA and the State Scientific and Technological Commission (SSTC) for their joint

financial sponsorship of the event with IOC. He then went on to outline the Marine Pollution Research and Monitoring functions within IOC and their link to future Global Ocean Observing System (GOOS) activities, in the context of the UN Conference on Environment and Development (UNCED) discussions. Of particular importance was the monitoring of coastal marine environments, where the methods to be investigated this week were especially relevant; he indicated that the Workshop was a Priority A activity in the Second Action Plan of IOC GIPME and he particularly welcomed its coming to fruition after a long planning period.

On behalf of the lecturing team, Dr Robert Clarke expressed their satisfaction in the location, accommodation and administrative (including computing) arrangements made for the workshop, all of which were very conducive to an intensive and successful activity - he added his thanks to the organizers and sponsors, and looked forward to a stimulating and productive week's work.

The Workshop itself then commenced with an introductory lecture, outlining the type and scale of problems at which the methods of analysis being presented were aimed. This was followed by a discussion session in which participants introduced themselves and the range of problems that they or their colleagues were investigating, and in what areas they expected enhanced progress as a result of the information gained from this workshop. The workshop then settled into a pattern of lectures, given in English without translation (but at as slow a pace as could be achieved), in which were sandwiched practical sessions in which genuine small-scale data sets were analyses on IBM-PC compatible computers, using the PRIMER software package. A detailed timetable of lectures and practical sessions is given in Annex II.

The full documentation for the Workshop, in most cases received by the participants in advance, consisted of a set of formal lecture notes for the methodological and interpretational aspects, a set of computer user notes for the PRIMER software, example sheets covering the data and questions for the practical sessions, and reprints of relevant literature, particularly charting the development and application of the abundance-biomass comparison(ABC) and multivariate (MDS-related) methods at the Plymouth Marine Laboratory, over the last few years. The full set of lecture notes for the 14 main lectures are reproduced in Annex III.

The final day of the workshop contained an opportunity to review the discussions and experiences of the week, in the context of what impact they might have on future use of these methods and software in the region, and also an invitation to the participants to fill in an anonymous questionnaire, on their reactions to the training course. The results of the latter are discussed in the next section. At a (less formal) closing ceremony, representatives of the organizers and participants expressed considerable satisfaction at the smooth-running of the workshop and its overall success. At the end, the participants were able to take away copies of the PRIMER programmes, on diskettes suitable for the machines at their own laboratories, and detailed instructions on how to get the software running (usually a straightforward exercise). All attendees were keen to take a copy and had, by the end of the week, been able to work through examples of all the main programmes in the package, in one or two instances on data that they themselves had brought to the workshop.

Finally, most participants were able to take up and enjoy the offer of a guided tour of the Third Institute buildings, including the mesocosm facility, and were also able to see a little of the attractive city and surroundings of Xiamen, thanks to a well-organized excursion on the afternoon of the fourth day.

4. EVALUATION

At the end of the Workshop, participants were asked to fill in the following questionnaire. The questions are given in full below, together with a summary of the replies (usually in the form of the percentage of replies that were a, b or c); all questionnaires were returned, completed in full.

QUESTIONNAIRE

Please complete the following questions, which will help us report back to IOC on the success or otherwise of the course. Please be honest, the answers are anonymous!

Q 1. Did the course announcement describe the content of the course:

a) well; b) adequately; c) poorly; (If c state why)

a) 100%; b) 0%; c) 0%

Q 2. Did you receive the package of lecture notes, computer manual and reprints:

a) in good time; b) too late to be useful; c) did not receive at all

a) 38%; b) 62%; c) 0%

Q 3. Were the general institute facilities:

a) good; b) average; c) poor (If c explain why)

a) 56%; b) 38%; c) 6%

Q 4. Were the computing facilities:

a) good; b) average; c) poor (If c explain why)

a) 56%; b) 44%; c) 0%

Q 5. Was the overall design of the course:

a) fine; b) too detailed; c) inadequate (If b or c explain why)

a) 94%; b) 0%; c) 6%

Q 6. Please give a score of 2 for good, 1 for average and 0 for poor for the content and ease of understanding of:

Lectures		Lecture Notes		Practicals		Computer Notes	
2:	100%	2:	88%	2:	75%	2:	56%
1:	0%	1:	12%	1:	25%	1:	44%
0:	0%	0:	0%	0:	0%	0:	0%

Q 7. Do the multivariate programmes fulfil the demands that you have for data analyses:

a) well; b) averagely; c) poorly (If c say why)

a) 82%; b) 18%; c) 0%

Q 8. Were the programmes:

a) easy to use; b) acceptable to use; c) difficult to use (If c say why)

a) 50%; b) 50%; c) 0%

Q 9. What difficulty did you have understanding the course language (English):

a) no real difficulty; b) some difficulty; c) a major problem

a) 50%; b) 44%; c) 6%

Q 10. Was the speed of progress through the course:

a) just right; b) too fast; c) too slow (If b or c say why)

a) 44%; b) 56%; c) 0%

Q 11. How does this course compare with others you have attended?

a) better than average; b) average; c) below average (If c) say why)

a) 82%; b) 18%; c) 0%

Q 12. Which parts of the course did you find most useful?

The most common reply (50%) was "all of it"! Three participants specified the practicals and programmes and the others identified specific lectures. There was no particularly common theme to the lectures mentioned - univariate and multivariate, statistical and interpretational aspects of the course were all singled out as being of importance.

Q 13. Briefly say for what sort of applications (if any) you might make use of the methods and software studied at the workshop.

The replies indicated that the methods were felt to be very relevant to pollution and more fundamental studies in the region. Some people specified particular types of pollution (oil-related, coastal construction) or geographical areas they were studying (Xiamen Bay, Fujian coast). Most talked about the type of communities they were studying (predominantly benthos, with a strong representation of meiobenthic work, but also some mention of phytoplankton, zooplankton and fish). People working specifically on shellfish or ecosystem studies also felt there were ideas here which would be of general benefit to them, and this even extended to general oceanographic work such as characterization of water masses. Only two participants did not say they were planning to use the methods or programmes in their own research, and one of those (whose job was in assessing rather than undertaking projects) felt they would be of benefit to a number of his colleagues and would pass the information and software on.

Q 14. What further activities (e.g. in WESTPAC) do you think should follow this workshop?

Comments were very diverse here, but one common suggestion (from 6 participants) was that this activity should be followed up in a couple of years time, after all attendees had been able to try out these methods exhaustively on their own problems, with a further workshop in which participants described their progress and discussed it with other WESTPAC colleagues and the lecturing team. Other suggestions were that regular correspondence should be established between participants, that IOC should provide other software and methods in the same way, and that ways be found of improving regular communication of new developments in this area to the region. There were also two requests for specific workshops, one particularly on oil-related problems and one on marine ecological modelling. More than one participant also remarked that this workshop was a good model for other WESTPAC activities and that such workshops be carried out more often.

Q 15. Are there any other comments that you wish to add?

The comments here were very gratifying to the organizers and lecturing team, with most people expressing a very warm thanks to IOC for the opportunity to participate, to the lecturing team for "excellent lectures" and to the Chinese hosts. The only recurring criticism (from 4 people) was that the workshop should have been longer, to allow more time to take in the "very rich content" and practice with computer operation (one participant suggested that next time it should be planned to last for 3 months to half a year!). A couple of participants also repeated their answers to the earlier Q2 that they would have liked to receive information earlier and that some prior information on financial arrangements and other practical matters of life in China for the non-Chinese participants would have been welcome. The only practical problem that was noted, however, by one participant was a little difficulty with the food (because of his religio-cultural background).

By any standards, the Workshop must be judged a considerable success. The overall response to the workshop was positive and enthusiastic. Course participants were generally very keen, prepared to work long and taxing days, and strived to take on board some complex concepts. In spite of the intensive nature of the course, it was notable that, after all participants had arrived (some of them had flight times which precluded them arriving at the start of the first day), not one person appeared to miss any of the lectures or practical sessions. Though the general level of previous experience of statistics and PC computing was low, and the facility with written and spoken English undoubtedly caused a little difficulty for several of the participants, nonetheless all appeared to feel that they had gained relevant knowledge and experience from the week. This is demonstrated by the rating of the overall course design (94% replied "fine" to Q5) and by the large number of replies to questions that gave the maximum possible rating. For all attendees and over all questions that had a straight multiple choice rating for aspects of the course, between:

a or 2):	good;	fine;	easy to use;	just right;	better than average etc.
b or 1):	adequate;	acceptable;	average		
c or 0):	poor;	below average;	inadequate;	difficult to use;	a major problem etc.

70% of the replies were a, 29% were b, with only 3 out of 224 replies of c. This is a very high level of satisfaction and indicates that the course was very worthwhile. Nonetheless, it is instructive to look more closely at the replies to specific questions.

Q1 on the accuracy of the course announcement attracted a 100% rating but it was a little more disappointing to note (Q2) that the package of lecture notes and associated workshop documentation were received too late to be useful in a majority of cases, in spite of the efforts made to prepare the material several months in advance. The difficulty here was caused by delays in receiving nominations for attendance and this suggests that planning for any future training workshop should seek to maximize the time between fixing the date of the workshop and the event itself. Due to various factors, the current workshop had a long lead time at the planning stage but the actual date of the workshop was only fixed 6 months previously; it may be that a slightly longer period would be optimal here.

The majority of participants gave the institute facilities (general and computing, Q3 and 4) top rating, with only one person expressing definite, though unspecified, dissatisfaction. The lecturing team too felt that the facilities and support available were excellent; in particular the computing equipment that was so essential to the course was very adequate. Six PCs had been requested for this number of participants but in fact 8 Pcs were provided, all with sufficient memory and ample disk space. This allowed 2 participants to every machine (an optimal number) and leaving flexibility to form groups of 3 (a viable number), allowing the practical sessions to continue unhindered during the occasional hardware failure. The one or two hardware problems encountered were fixed within hours by the Third Institute support personnel. There were also no significant problems on the software side. The PRIMER package was mounted on all machines within a couple of hours. It was noted that a couple of the machines contained a common Western "virus", showing the extent to which this is now a worldwide phenomena; however, this caused no difficulties during the Workshop and great care was taken not to transmit the virus to other machines or to the floppy disks containing the PRIMER package that participants took home with them. Few machines were fitted with math co-processors - this is not a requirement of the PRIMER package but it is heavily compute-intensive software and will run an order of magnitude faster where such boards are fitted. Some participants may therefore have felt the software to be a little slow in execution, which is possibly why nearly half considered the computing facilities to be "average" rather than "good". Overall though, the problems anticipated by the Third Institute in providing the computing facilities, when this workshop was first considered some time ago, had entirely dissipated; this reflects the immense speed of development that is apparent throughout Xiamen.

On the subject of arrangements within Xiamen, the questionnaire did not specifically ask about the accommodation and other administrative arrangements but, as far as the lecturers were concerned, these were also excellent. The rooms and facilities at the International Academic Exchange Center of Xiamen University were more than acceptable and the food of excellent quality. The local administration was efficient and thorough, and the hospitality, including a final workshop dinner, was warm and much appreciated. The local organizing committee clearly did everything in its power to make sure that the event proceeded smoothly and successfully and they are to be thanked and congratulated on their efforts.

On the specific content of the workshop (Q5 - Q8) participants gave uniformly high ratings, particularly on the overall design of the course, the content of the lectures and the relevance of the multivariate programmes to their data analysis problems. In spite of the fact that several participants had no previous experience of Pcs or elementary DOS operations, no-one rated the programmes difficult to use - opinion was equally divided between "easy to use" and "acceptable to use".

Teaching the course entirely in English, without translation, was expected to provide some difficulties and the reply to Q9 shows that half the participants did experience some problems, though only one had a major problem. To some extent, also, the lecturers felt that the more complex ideas and interpretations were difficult to get across because of the language barrier. Inevitably, the lectures were given at a very slow and measured pace, and some of the prepared material was omitted. However, the important ideas and their practical application were all covered, and the participants will be able to follow up some of the supplementary material at a later stage because it is available to them both in the lecture notes and in a number of literature reprints that were distributed. In spite of the efforts made to simplify the presentation of the material and to slow the pace of exposition, just over half the participants considered the speed of progress through the course (Q10) to be too fast. This is not really surprising because it reflects both the language difficulty and the lack of prior exposure to Pcs, in addition to the inherently demanding nature of these methodological developments. Clearly, a number of participants would have preferred a much longer workshop (including the person who specified 3 to 6 months!) but this was not a realistic possibility and one suspects that, in this area, whatever time had been set aside would have been considered insufficient. The point remains that the participants now have the ideas, the literature and, most importantly, the tools (the programmes) to pursue the application of these methods further, if they have the incentive and ability to do so. That a great many of them do is evidenced by the answers to the earlier questions, that the material of the workshop was of great relevance and effectively presented, and by the comments made in answer to Q12-15 (summarized earlier). Community data sets arise in a range of scientific studies in the WESTPAC area, both in pollution-related and more fundamental research, and it is clear that modern analysis tools are not widely available in the region. It would appear also that the necessity for access of biologists to appropriate computing hardware is only just being appreciated, and it is hoped that in a small way this workshop will have opened one specific avenue for further expansion, as well as fostered research links both to the WESTPAC region and within it.

ANNEX I

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ANNEX II

TIMETABLE

Monday - October 19

08:30-09:30	Opening Ceremony and Introduction (IOC/GEEP, SSTC and SOA)
09:30-10:45	Lecture 1, A framework for studying changes in community structure (Dr Warwick)
10:45-11:00	Tea/Coffee break
11:00-12:15	Participants describe their main research areas and discuss community data sets arising in pollution effects studies in their WESTPAC regional programmes.
12:15-13:45	Lunch break
13:45-14:30	Lecture 2, Measures of similarity of species abundance/biomass between samples (Dr Clarke)
14:30-15:30	Lecture 3, Hierarchical clustering (Dr Clarke)
15:30-15:45	Tea/Coffee break
15:45-16:15	Introduction to PRIMER programmes and format for data entry (Mr Carr)
16:15-18:15	Practical session on lectures 2 and 3

Tuesday - October 20

08 30-10:15	Lecture 4, Ordination of samples by Principal Components Analysis (Dr Clarke)
10:15-10:30	Tea/Coffee break
10:30-12:15	Lecture 5, Ordination of samples by Multi-Dimensional Scaling (MDS) (Dr Clarke)
12:15-13:45	Lunch break
13:45-15:30	Practical session on lectures 4 and 5
15:30-15:45	Tea/Coffee break
15:45-17:00	Testing for differences between groups of samples (Dr Clarke)
17:00-18.15	Practical session on lecture 6

Wednesday - October 21

08:30-09:45	Lecture 7, Species analyses (Dr Clarke)
09:45-10:30	Practical session on lecture 7
10:30-10:45	Tea/Coffee break
10:30-10:45	Tea/Coffee break

10:45-12:15	Lecture 8, Diversity measures, dominance curves and other graphical analyses (Dr Warwick)
12:15-13:45	Lunch break
13:45-14:45	Practical session on lecture 8
14:45-15:30	Lecture 9, Transformations (Dr Clarke)
15:30-15:45	Tea/Coffee break
15:45-16.45	Lecture 10, Species aggregation; comparison of impact studies from different regions (Dr Warwick)
16.45-18:15	Practical session on lectures 9 and 10 (or analysis of "own" data sets, where brought to the workshop)

Thursday - October 22

08.30-09:30	Lecture 11, Linking community analyses to environmental variables (Dr Clarke)
09.30-10.30	Practical session on lecture 11
10.30-10:45	Tea/Coffee break
10:45-11.30	Lecture 12, Causality: community experiments in the field and laboratory (Dr Warwick)
11:30-12.15	Lecture 13, Data requirements for biological effects studies: which components and attributes of the biota to examine? (Dr Warwick)
12:15-13:45	Lunch break
13.45-18.15	Visit to Gulangyu Isle

Friday - October 23

08.30-10:00	Lecture 14, Relative merits of univariate, graphical and multivariate techniques (Dr Warwick)
10:00-10:15	Tea/Coffee break
10.15-11.45	Final discussion (including relevance of this methodology to community data sets from the WESTPAC region and suggestions for future activities); participants complete questionnaires.
11:45-12:15	Closing Ceremony (IOC/GEEP, SSTC and SOA)
12:15-13:45	Lunch break
13:45-15.30	Details of mounting PRIMER programmes and entering data on participants' machines (Mr Carr)
15:30-15:45	Tea/Coffee break
15:45-17:00	Guided tour of the 3rd Institute of Oceanography
17:00-18:00	Workshop software distributed to participants

ANNEX III

LECTURE NOTES

The lecture notes in this annex expound a coherent framework for the analysis and interpretation of multivariate community data, typically large matrices of counts (or biomass) of many species from samples taken at different sites or times. The material concentrates on a suite of statistical and graphical techniques exploited and developed at the Plymouth Marine Laboratory (PML), UK, over the last decade. Their utility in describing communities and identifying patterns of change, in natural or pollution impact situations, has been successfully demonstrated in a wide range of published papers (see the papers involving Carr, Clarke or Warwick in the bibliography). The main emphasis is on benthic community studies in estuarine and coastal zone sediments, though the statistical and graphical methods have wider applicability.

The Workshop supplements these lectures with practical sessions, involving the analysis of example community data sets from the published literature and using the suite of IBM PC programmes developed at the Plymouth Marine Laboratory (the PRIMER package:- Plymouth Routines In Multivariate Ecological Research). The lectures are designed for biologists who collect and need to analyses community data in monitoring or environmental impact studies. They assume a rudimentary knowledge of standard statistical concepts of variation and hypothesis testing but no formal statistical background is necessary: the multivariate methods are described from first principles and the advocated non-parametric approach lends itself to straightforward, non-technical explanations of how and why the methods work. The lectures are as follows:

No. 1	A framework for studying changes in community structure.
No. 2	Measures of similarity of species abundance/biomass between samples.
No. 3	Hierarchical clustering.
No. 4	Ordination of samples by Principal Components Analysis (PCA).
No. 5	Ordination of samples by Multi-Dimensional Scaling (MDS).
No. 6	Testing for differences between groups of samples.
No. 7	Species analyses.
No. 8	Diversity measures, dominance curves and other graphical analyses.
No. 9	Transformations.
No. 10	Species removal and aggregation.
No. 11	Linking community analyses to environmental variables.
No. 12	Causality: community experiments in the field and laboratory.
No. 13	Data requirements for biological effects studies: which components and attributes of the biota to examine?
No. 14	Relative merits of univariate, graphical and multivariate techniques.

LECTURE 1

A FRAMEWORK FOR STUDYING CHANGES IN COMMUNITY STRUCTURE

1 STAGES

1. REPRESENTING COMMUNITIES (graphical description of faunal relations).

2. DISCRIMINATING SITES on the basis of faunal composition (e.g. spatial: control v. impacted, temporal: before v. after impact).

3. DETERMINING LEVELS OF "STRESS" or disturbance in communities.

4. LINKING WITH ENVIRONMENTAL VARIABLES (e.g. correlating to contaminants)

5. ESTABLISHING CAUSALITY of link to contaminants.

2 TECHNIQUES

UNIVARIATE	-	diversity indices
	-	indicator species abundance
DISTRIBUTIONAL	-	"ABC" curves (k-dominance)
	-	distn. of individuals amongst species
MULTIVARIATE	-	triangular matrix of similarities between samples, leading to:
		- hierarchical classification (CLUSTER)
		- multidimensional scaling (MDS)
	-	principal component analysis (PCA)

3 UNIVARIATE TECHNIQUES

STAGES		EXAMPLES	
		Diversity indices	Indicator species
1)	REPRESENTING COMMUNITIES	Means ± confidence intervals (CIs) for each site	
2)	DISCRIMINATING SITES	One-way analysis of variance (ANOVA)	
3)	DETERMINING STRESS LEVELS	By reference to historical data, e.g. ultimately a decrease in diversity	initial increase in "opportunist" species
4)	LINKING TO ENVIRONMENT	Regression techniques	
5)	ESTABLISHING CAUSALITY	Mesocosm or field experiments with controlled dosing of contaminants. All entries above apply, e.g. now significant discrimination of "sites" (=treatments) demonstrates that contaminant causes biological effect.	

IOC/GEEP WORKSHOP ON BIOLOGICAL EFFECTS OF POLLUTANTS

OSLO 1986: MACROFAUNAL DATA, Gray et al. (1988)

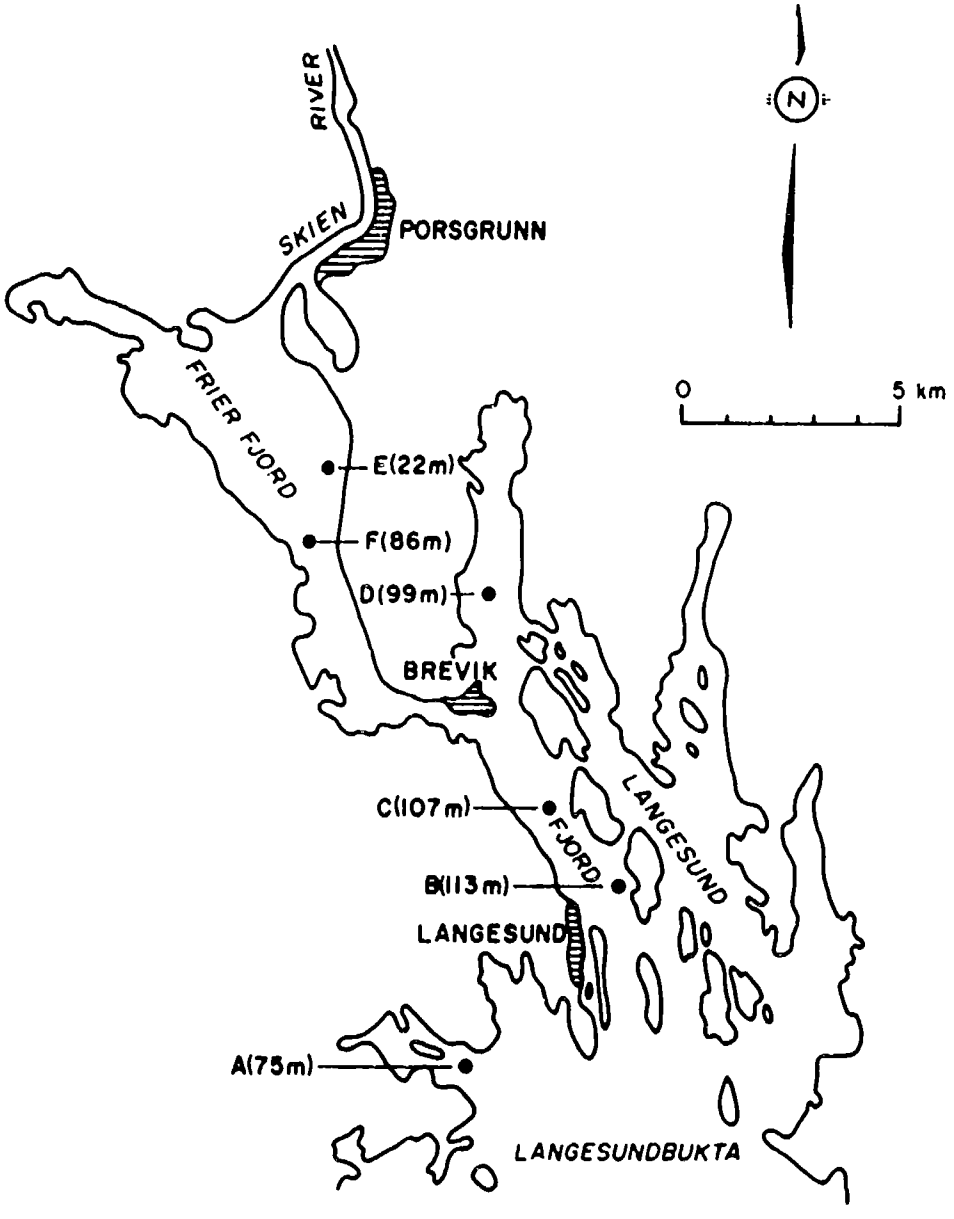


Fig. 1.1. *Frierfjord and Langesundfjord, Norway. Benthic community sampling sites (A-G) for the Oslo Workshop.*

FOUR 0.1M² DAY GRAB SAMPLES TAKEN AT 6 SITES (A-E,G), SIEVED AT 1MM, AND COUNTS/BIOMASS RECORDED OF 110 SPECIES IDENTIFIED.

Species	A				B			
Cerianthus lloydi	0	0	0	0	0	0	0	0
Halicryptus sp.	0	0	0	1	0	0	0	0
Onchnesoma	0	0	0	0	0	0	0	0
Phascolion strombi	0	0	0	1	0	0	1	0
Golfingia sp.	0	0	0	0	0	0	0	0
Holothuroidea	0	0	0	0	0	0	0	0
Nemertina, indet.	12	6	8	6	40	6	19	7
Polychaeta, indet.	5	0	0	0	0	0	1	0
Amaena trilobata	1	1	1	0	0	0	0	0
Amphicteis gunneri	0	0	0	0	4	0	0	0
Ampharetidae	0	0	0	0	1	0	0	0
Anaitides groenlandica	0	0	0	0	1	0	0	0
Anaitides sp.	0	0	0	0	0	0	0	0

Table 1.1. Macrofaunal abundance matrix (part), numbers per 0.1m².

Species	A				B			
Cerianthus lloydi/10	0	0	0	0	0	0	0	0
Halicryptus sp.	0	0	0	26	0	0	0	0
Onchnesoma	0	0	0	0	0	0	0	0
Phascolion strombi	0	0	0	6	0	0	2	0
Golfingia sp.	0	0	0	0	0	0	0	0
Holothuroidea	0	0	0	0	0	0	0	0
Nemertina, indet./10	1	41	391	1	5	1	2	1
Polychaeta, indet.	9	0	0	0	0	0	0	0
Amaena trilobata	144	14	234	0	0	0	0	0
Amphicteis gunneri	0	0	0	0	45	0	0	0
Ampharetidae	0	0	0	0	0	0	0	0
Anaitides groenlandica	0	0	0	7	11	0	0	0
Anaitides sp.	0	0	0	0	0	0	0	0

Table 1.2. Macrofaunal biomass matrix (part), mg per 0.1m².

UNIVARIATE: REPRESENTATION AND DISCRIMINATION

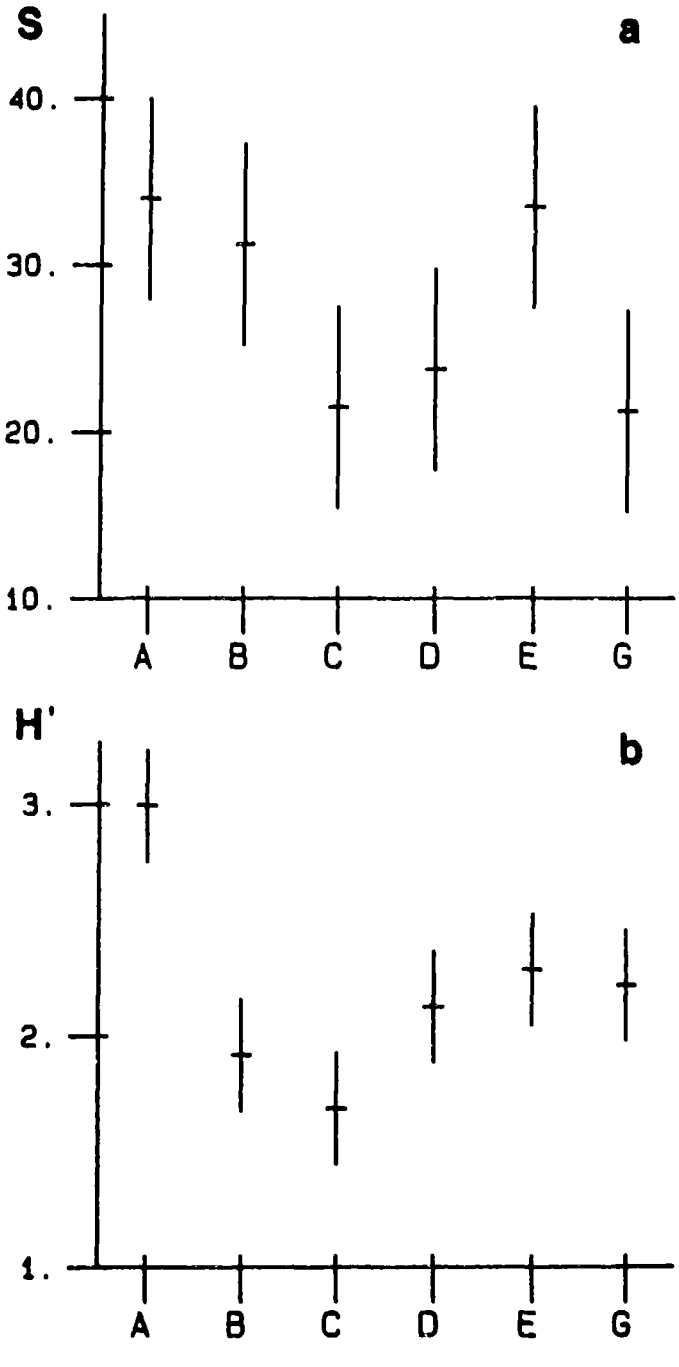


Fig 1.2. Frierfjord macrofauna. Means and 95% confidence intervals for two indices.
a) Number of species (S); b) Shannon diversity (H').

DISTRIBUTIONAL TECHNIQUES

EXAMPLES

"ABC" curves (k-dominance curves)	Distribution of individuals amongst species

STAGES

1) REPRESENTING COMMUNITIES	Curves for each site (or prerefably replicate)
2) DISCRIMINATING SITES	ANOSIM (Analysis of Similarities) test on "distances" between every pair of curves
3) DETERMINING STRESS LEVELS	Biomass curve drops below numbers curve when subject to disturbance | Species abundance distribution is less "smooth" with disturbance
4) LINKING TO ENVIRONMENT	Possible for univariate summary statistics by regression.
5) ESTABLISHING CAUSALITY	Mesocosm or field dosing experiments. Entries above apply.

ORGANIC ENRICHMENT OF BENTHOS - Pearson (1975)

LOCH LINNHE (SCOTLAND) MACROFAUNA -
discharges started in 1966, increased 1970, decreased 1972.

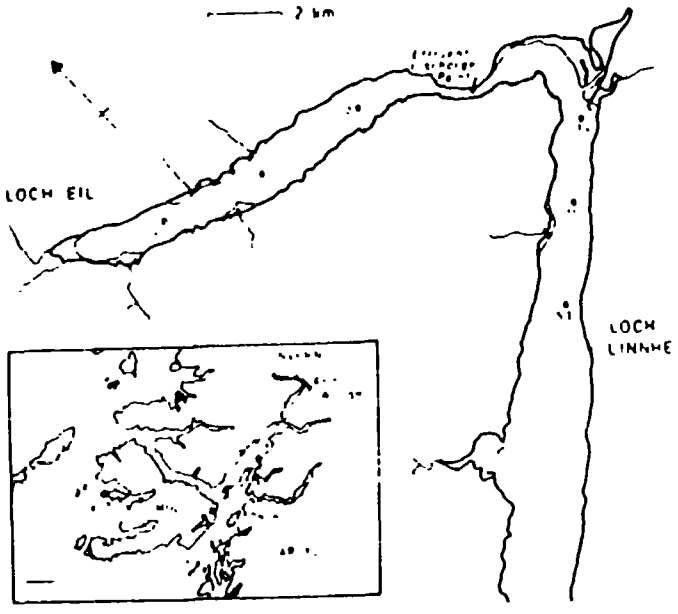


Fig. 1.3. Loch Linnhe and Loch Eil, showing site 34, sampled over 1963-1973.

Species	1963		1964		1965		1966	
	No.	Wt	No.	Wt	No.	Wt	No.	Wt.
Mollusca								
Scalopus ventrolineatus Salvini-Plawen	-	-	-	-	11	0.05	-	-
Nucula tenuis (Montagu)	2	0.01	13	0.07	16	0.10	6	0.04
Mytilus edulis L.	-	-	-	-	5	0.09	-	-
Modiolus sp. indet.	-	-	-	-	-	-	-	-
Thyasira flexuosa (Montagu)	93	3.57	210	7.98	28	1.06	137	5.17
Myrtea spinifera (Montagu)	214	27.39	136	17.41	2	0.26	282	36.10
Lucinoma borealis (L.)	12	0.39	26	1.72	-	-	22	0.73
Montacuta ferruginosa (Montagu)	1	0.00	-	-	4	0.02	-	-
Mysella bidentata (Montagu)	-	-	-	-	-	-	-	-
Abra sp. indet.	-	-	-	-	12	0.26	-	-
Corbula gibba (Olivi)	2	0.13	8	0.54	9	0.27	2	0.13
Thracia sp. indet.	-	-	-	-	-	-	-	-

Table 1.3. Numbers/biomass matrix (part) for site 34 - one (pooled) set of values per year (1963-1973).

DISTRIBUTIONAL : REPRESENTATION AND STRESS DETERMINATION

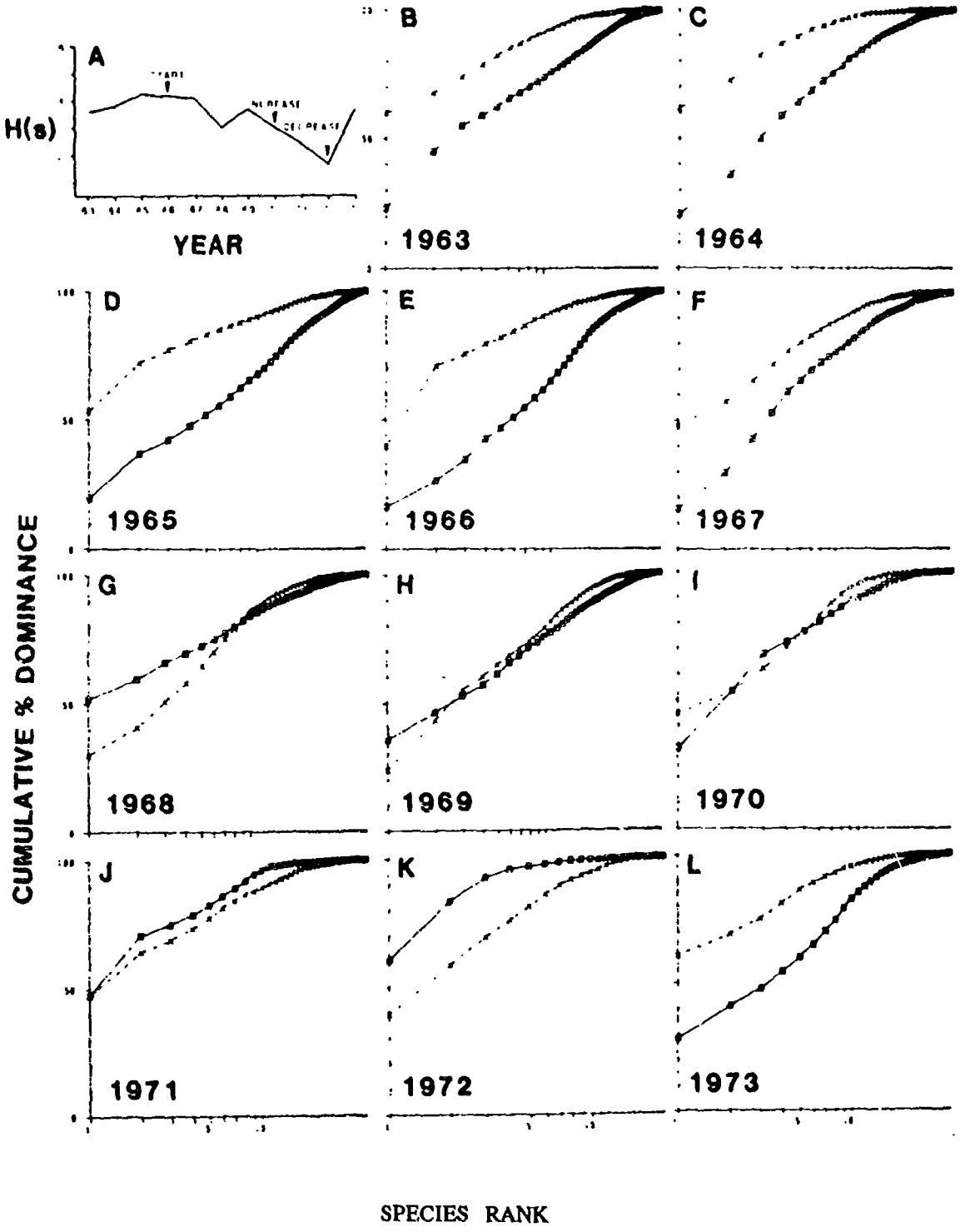
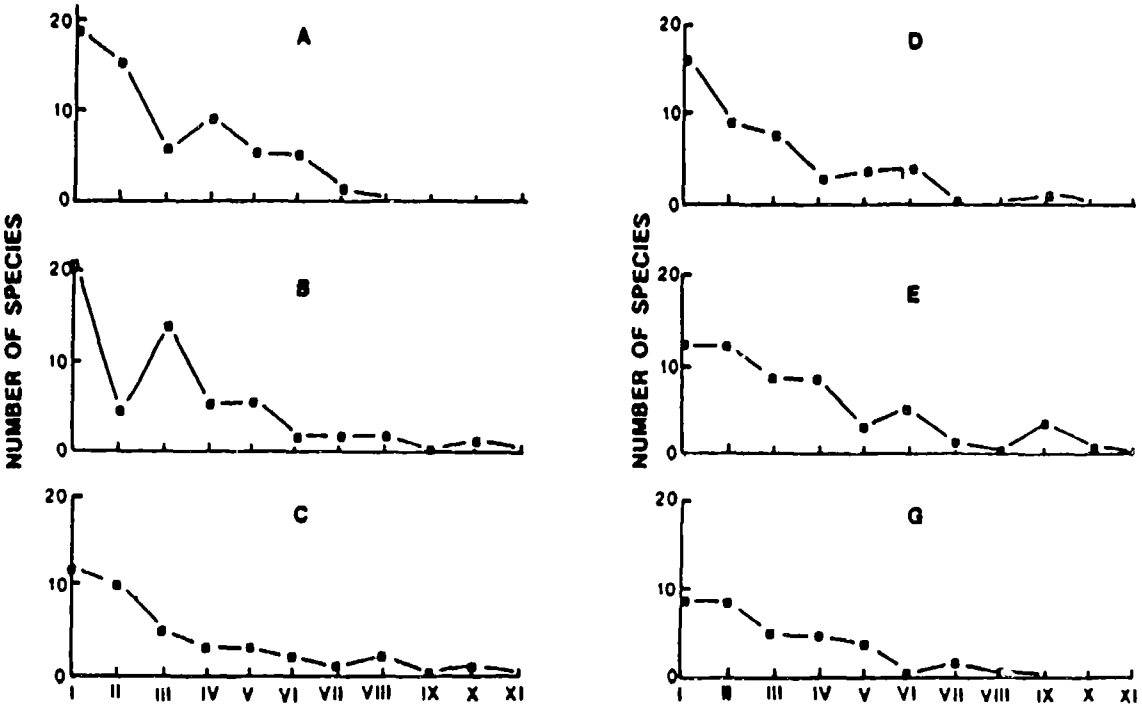


Fig. 1.4. Loch Linnhe site 34. (A) Shannon diversity.

(B)-(L) ABC curves for 1963-73 : biomass (x), numbers ( ). Warwick (1986).

DISTRIBUTIONAL : REPRESENTATION AND STRESS DETERMINATION



NUMBER OF INDIVIDUALS PER SPECIES (X2 GEOMETRIC CLASSES)

Fig. 1.5. Frierfjord macrofauna, sites A-E,G.

Number of species against number of individuals per species in geometric classes (I = 1 individual per species, II = 2-3 ind. per spp., III = 4-7, IV = 8-15 etc.). Gray et al. (1988).

MULTIVARIATE TECHNIQUES

	EXAMPLES		
	Hierarchical clustering	MDS ordination	PCA ordination
STAGES			
REPRESENTING COMMUNITIES	Dendrogram of replicates	Configuration of replicates (often 2-D)	
DISCRIMINATING SITES	ANOSIM test on triangular matrix of similarities. Similarity percentage breakdown (SIMPER) gives species responsible.		Multinormal tests (e.g. Wilks' K), but often invalid.
DETERMINING STRESS LEVELS	Not appropriate		
LINKING TO ENVIRONMENT	Visual (superimposing environmental variables on faunal ordinations). Finding subset of environmental variables whose ordination "best" matches the faunal ordination.		
ESTABLISHING CAUSALITY	Mesocosm or field dosing experiments. Use above techniques - significance in discriminating "sites" (= treatments) establishes causality		

MULTIVARIATE: REPRESENTATION

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
A1	-											
A2	61	-										
A3	69	60	-									
A4	65	61	66	-								
B1	37	28	37	35	-							
B2	42	34	31	32	55	-						
B3	45	39	39	44	66	66	-					
B4	37	29	29	37	59	63	60	-				
C1	35	31	27	25	28	56	40	34	-			
C2	40	34	26	29	48	69	62	56	56	-		
C3	40	31	37	39	59	61	67	53	40	66	-	
C4	36	28	34	37	65	55	69	55	38	64	74	-

Table 1.4. Frierfjord macrofauna counts. Similarities (Bray-Curtis coefficient, after $\sqrt{\sqrt{}}$ transformation) between every pair of replicates (sites A-C only).

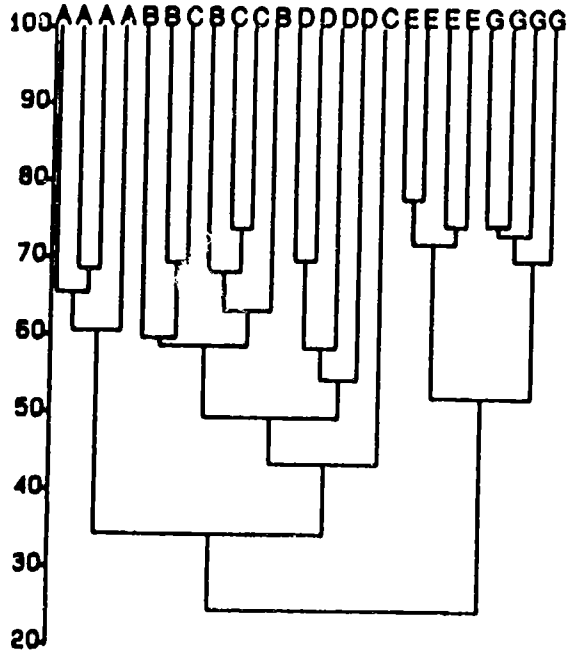


Fig. 1.6. Frierfjord macrofauna. Dendrogram for hierarchical clustering (group-average link) of 4 replicates from 6 sites, using above similarities.

MULTIVARIATE : REPRESENTATION AND DISCRIMINATION

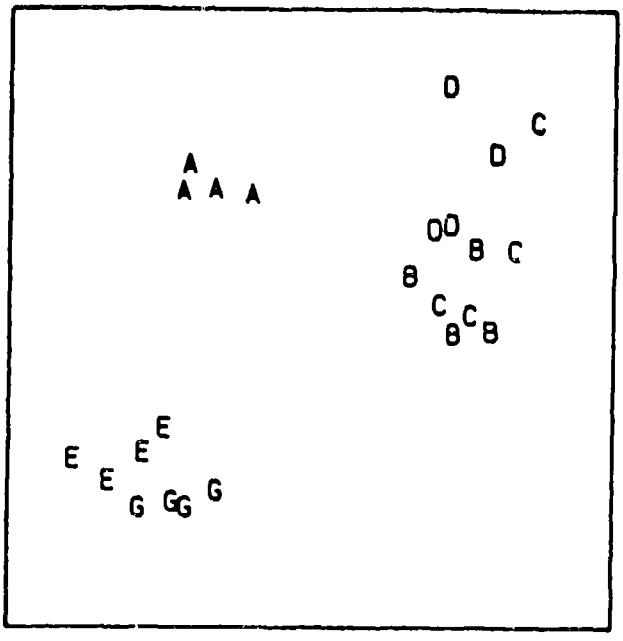


Fig. 1.7. Frierfjord macrofauna. Non-metric MDS ordination (in 2-D) of the 4 replicates from each of sites A-E and G, from Table 1.4 similarities.

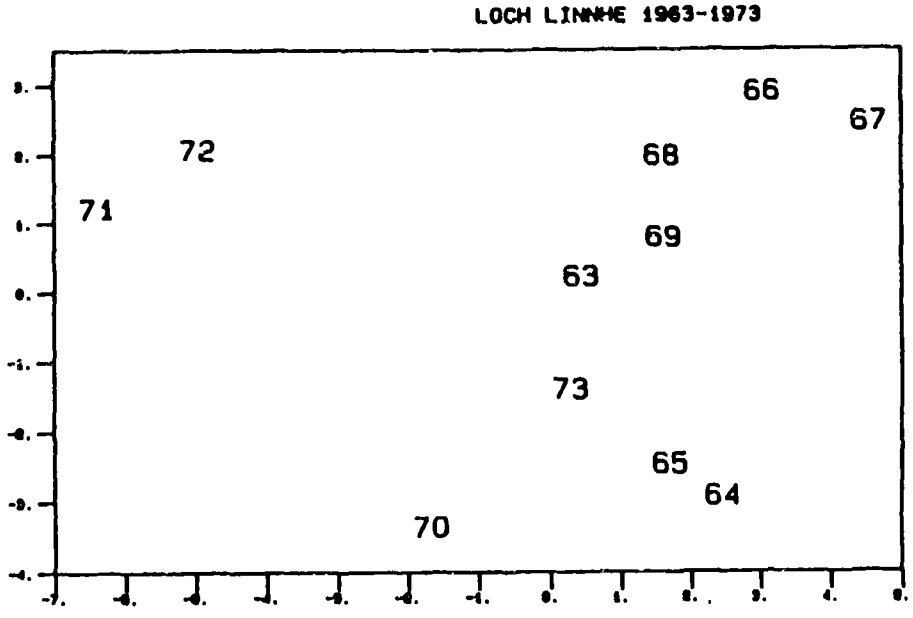


Fig. 1.8. Loch Linnhe macrofauna. PCA ordination (in 2-D) of the 11 years abundance data, omitting the less-common species.

MULTIVARIATE : LINKING TO ENVIRONMENT

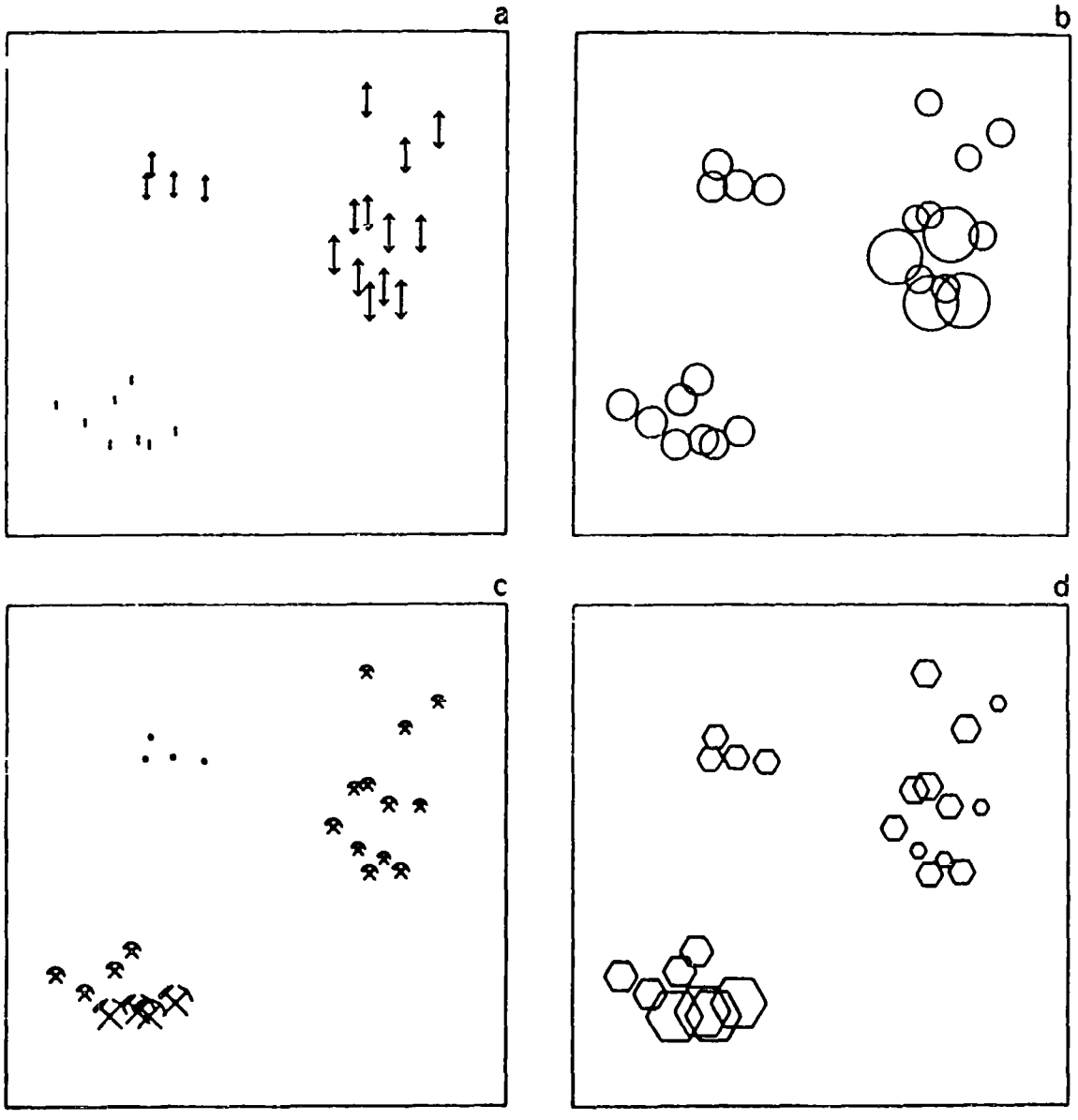


Fig. 1.9. Frierfjord macrofauna. Values of four environmental variables: (a) water depth, (b) sediment grain size, (c) metal and (d) PAH concentrations in sediment, superimposed on the abundance-based MDS.

NUTRIENT ENRICHMENT MESOCOSM EXPERIMENT
- Gee et al.(1985)

Meiofaunal abundances under 2 dosing regimes, Solbergstrand facility (NIVA), Norway

	Control				Low dose				High dose			
	C1	C2	C3	C4	L1	L2	L3	L4	H1	H2	H3	H4
Copepoda, Harpacticoida												
Ectinosomidae												
Halectinosoma gothiceps	-	-	1	1	16	23	8	16	-	1	-	-
Tachidiidae												
Danielssania fusiformis	1	1	1	1	1	3	8	5	1	-	-	3
Tisbidae												
Tisbe sp. 1 (gracilis group)	-	-	-	-	-	-	-	-	2	27	119	31
Tisbe sp. 2 (graciloides?)	-	-	-	-	45	22	39	25	6	-	3	32
Tisbe sp. 3	-	-	-	-	86	83	88	-	5	29	-	20
Tisbe sp. 4	-	-	-	-	151	249	264	87	8	-	-	34
Tisbe sp. 5	-	-	-	-	129	-	-	115	4	-	1	40
Diosaccidae												
Typhlamphiascus typhlops	4	2	2	4	5	8	4	3	-	-	-	-
Bulbamphiascus imus	1	-	-	2	-	-	-	-	-	-	-	-
Stenhelia reflexa	3	1	-	1	2	-	-	-	-	-	-	-
Amphiascus tenuiremis	1	-	-	-	-	-	2	6	-	-	-	-
Ameiridae												
Ameira parvula	-	-	-	-	4	2	3	2	2	-	1	2
Proameira simplex	-	-	-	-	-	2	-	5	-	-	-	-
Paramesochridae												
Leptopsyllus paratypicus	-	-	1	-	-	-	-	-	-	-	-	-
Cletodidae												
Enhydrosoma longifurcatum	2	2	1	2	3	1	-	-	-	-	-	-
Laophontidae												
Unidentified copepodite	-	-	-	-	-	-	1	-	-	-	-	-
Ancorabolidae												
Ancorabolis mirabilis	3	-	4	4	2	18	3	3	27	3	1	-
Unidentified												
Copepodites	-	-	1	-	1	1	1	3	-	1	-	-

Table 1.5. Copepod numbers (nematodes not shown) from 4 boxes for each treatment (high, low and no additions of powdered Ascophyllum nodosum).

MULTIVARIATE : ESTABLISHING CAUSALITY

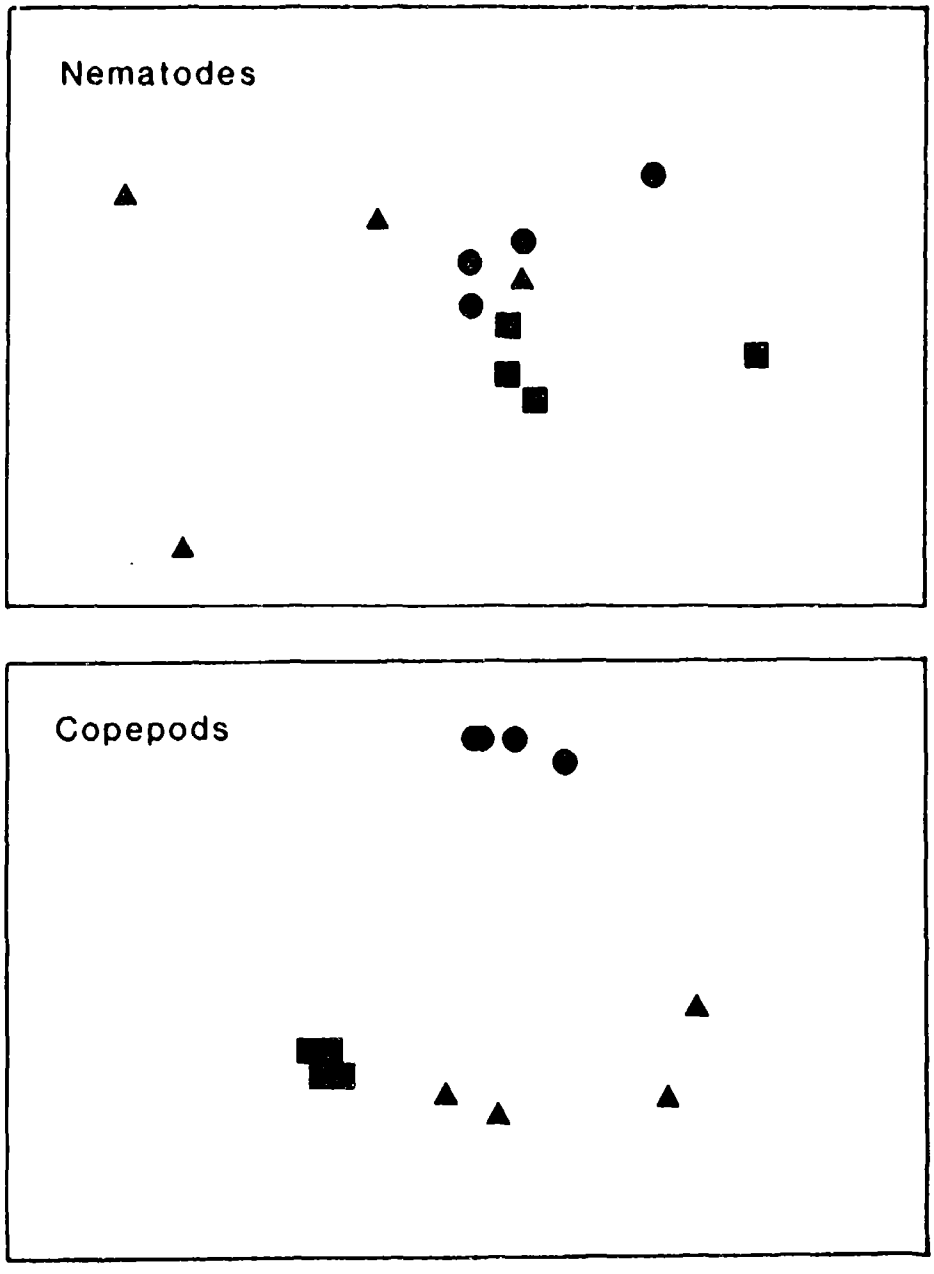


Fig. 1.10. Mesocosm meiofauna (nutrient enrichment).
MDS ordination of abundances from 4 replicate boxes from 3 treatments:
circles = control, squares = low dose, triangles = high dose.
(Gee et al., 1985).

DATA TRANSFORMATION AND SPECIES SELECTION/AGGREGATION

Some techniques may need TRANSFORMATION of the raw abundances/biomass (or derived statistics) for:

a) validity of assumptions for statistical analysis (e.g. normality, constant variance);

b) balancing contributions of rare/abundant species.

Some techniques may be possible with data on SELECTED (more dominant) species or data AGGREGATED to higher taxonomic levels, thus minimising identification time.

TECHNIQUE	EXAMPLES	TRANSFORMATION	SELECTION/ AGGREGATION
UNIVARIATE	Diversity indices	Counts: No Index: Possibly	No
	Indicator species	Yes (on counts /biomass)	Yes
DISTRIBUTIONAL	ABC curves	Possible but not usual	Possible
	Ind. among species	No	No
MULTIVARIATE	Cluster	Usual (log or 4th root) on counts/biomass. MDS transforms similarities also, to ranks.	Possible
	MDS		Possible
	PCA		Needed

LECTURE 2

MULTIVARIATE METHODS: MEASURES OF SIMILARITY OF SPECIES ABUNDANCE/BIOMASS BETWEEN SAMPLES

DATA MATRIX: A p (species) x n (samples) array of scores (counts or biomass). The n samples might consist of a number of replicates (possibly only one) at each of a number of sites or times.

SIMILARITY COEFFICIENT:

Measures the similarity (S) of the community structure between any pair of samples (thus SAMPLE SIMILARITIES), using:

a) absolute numbers (or biomass) of each species,

b) relative numbers (or biomass), i.e. STANDARDISE the scores, to reflect only species COMPOSITION (%),

c) only presence or absence of each species.

S is usually defined in the range (0,1) or (0,100%).

S = 1 (or 100%) means samples are totally similar,

S = 0 means samples are totally dissimilar.

SIMILARITY MATRIX:

This is a set of similarity coefficients, calculated between every pair of samples and laid out in a lower triangular array.

Similarity matrices are the basis for many clustering and ordination techniques (REPRESENTATION) and associated tests (DISCRIMINATION), which:

a) discriminate sites or times (similarities between replicates at a site > similarities between sites)

b) cluster sites (similarities within groups of sites > similarities between groups)

c) allow gradation of sites (site A has similarities with B, and B has with C, but A and C less similar)

SPECIES SIMILARITY MATRIX:

A matching triangular array of similarities between every pair of species, in terms of patterns of occurrence across the samples.

Many different ways to assess similarity (because data is multi-species). One of most useful in ecology is:

BRAY-CURTIS COEFFICIENT: (Bray and Curtis 1957).
Similarity between jth and kth samples is:

$$S_{jk} = 100\left(1 - \frac{\Sigma_{i=1}^{p} |y_{ij} - y_{ik}|}{\Sigma_{i=1}^{p} (y_{ij} + y_{ik})}\right) = 100 . \frac{\Sigma_{i=1}^{p} 2 \min(y_{ij}, y_{ik})}{\Sigma_{i=1}^{p} (y_{ij} + y_{ik})} \qquad (2.1)$$

where y_{ij} = score (count or biomass) for ith species in jth sample ($i = 1,2,\ldots,p$; $j=1,2,\ldots,n$).

Example: Loch Linnhe macrofauna (Pearson 1975).

(a)

Year:	64	68	71	73
(Sample:	1	2	3	4)
Species				
Echinoca.	9	0	0	0
Myrioche.	19	0	0	3
Labidopl.	9	37	0	10
Amaeana	0	12	144	9
Capitella	0	128	344	2
Mytilus	0	0	0	0

(b)

Sample	1	2	3	4
1	-			
2	8	-		
3	0	42	-	
4	39	21	4	-

Table 2.1. (a) Abundance (untransformed) for some selected species and years from site 34 data.
(b) Resulting Bray-Curtis similarity matrix.

1) Note S = 0 if the two samples have no species in common (e.g. 1 and 3 above).
2) A scale change in y (e.g. biomass changed from mg per m^2 to per cm^2) does not change S.
3) "Joint absences" also have no effect on S (as is desirable), e.g. can omit species 6 in the table.

With "raw" counts (or biomass), S gives too much weight to large scores, so a $\log(1+y)$ or $\sqrt{\sqrt{y}}$ transform is often applied, before computing S.

Example: Loch Linnhe macrofauna, $\sqrt{\sqrt{}}$ transformation

(a)

Year:	64	68	71	73
(Sample:	1	2	3	4)
Species				
1	1.7	0	0	0
2	2.1	0	0	1.3
3	1.7	2.5	0	1.8
4	0	1.9	3.5	1.7
5	0	3.4	4.3	1.2
6	0	0	0	0

(b)

Sample	1	2	3	4
1	-			
2	26	-		
3	0	68	-	
4	52	68	42	-

Table 2.2. (a) transformed abundances for 4 years.
(b) Resulting Bray-Curtis similarity matrix.

CANBERRA COEFFICIENT: Lance and Williams 1967.

Similarity between samples j and k is:

$$S_{jk} = 100(1 - p^{-1} \Sigma_{i=1}^{p} \frac{|y_{ij} - y_{ik}|}{(y_{ij} + y_{ik})}) \qquad (2.2).$$

It gives a more equal contribution from each species (so tends to be overdominated by rarer ones).

CORRELATION COEFFICIENT: Product-moment correlation

$$r_{jk} = \Sigma_i (y_{ij} - \bar{y}_{.j})(y_{ik} - \bar{y}_{.k}) / \sqrt{[\Sigma_i (y_{ij} - \bar{y}_{.j})^2 . \Sigma_i (y_{ik} - \bar{y}_{.k})^2]} \qquad (2.3)$$

is not a similarity (it can be <0). Use:

$$S_{jk} = 50 (1 + r_{jk}) \qquad (2.4),$$

but note that S increases with more joint absences.

PRESENCE/ABSENCE DATA

Many similarity coefficients have been proposed based on (0,1) data arrays (Sneath and Sokal 1973). For comparing samples j and k let:

a	=	number of species present in both samples,
b+c	=	number present in one sample and not the other,
d	=	number absent from both samples.

"SIMPLE MATCHING" COEFFICIENT:

$$S_{jk} = 100.(a+d)/(a+b+c+d) \qquad (2.5)$$

Note that this is a function of joint absences (d).

JACCARD'S COEFFICIENT:

$$S_{jk} = 100.a/(a+b+c) \qquad (2.6)$$

SORENSEN (OR DICE) COEFFICIENT:

$$S_{jk} = 100.2a/(2a+b+c) \qquad (2.7)$$

This is simply BRAY-CURTIS applied to (0,1) data.

McCONNAUGHEY COEFFICIENT (McConnaughey 1964):

$$S_{jk} = 100[a(2a+b+c)]/[2(a+b)(a+c)] \qquad (2.8)$$

RECOMMENDATION:

1) Use coefficient not dependent on joint absences.

2) Similarities from raw counts (or biomass) are too dominated by common (or large) species, but

3) reduction to presence/absence loses too much useful information, so recommend use:

4) BRAY-CURTIS on $\sqrt{\sqrt{y}}$ or $\log(1+y)$ transformed data.

5) Standardize scores if non-comparable sample volumes used, or if "patchiness" makes compositional change more relevant than fluctuations in absolute counts.

SPECIES SIMILARITIES: These are computed from the same data array but between any pair of species (rows i,l say) across all samples (columns).

BRAY-CURTIS: $$S'_{il} = 100\left(1 - \frac{\Sigma_{j=1}^{n} |y_{ij} - y_{lj}|}{\Sigma_{j=1}^{n} (y_{ij} + y_{lj})}\right) \qquad (2.9)$$

However: 1) Similarities between rare species have little meaning (S' usually 0) and should be omitted from any species clustering or ordination.

2) Standardization (not transformation) of y needed:

$$y^{*}_{ij} = 100\ y_{ij}/(\Sigma_{k=1}^{n}\ y_{ik}) \qquad (2.10)$$

(before computing S'), so two species in strict ratio across samples are "perfectly similar".

Example — Counts — Similarities

Sample:	1	2	3	4	5		Species	1	2	3
Species							1	-		
1	2	0	0	4	4	→	2	33	-	
2	10	0	0	20	20		3	20	7	-
3	0	4	4	1	1					
		↓	Standardize							
Species							1	-		
1	20	0	0	40	40	→	2	100	-	
2	20	0	0	40	40		3	20	20	-
3	0	40	40	10	10					

CORRELATION coefficients are more appropriate for species similarity, since they incorporate scale changes, but the location changes are undesirable.

RECOMMENDATION: For species similarities, use BRAY- CURTIS on standardised scores. Remove rarer species (never >3%, say, of total score in any sample).

DISSIMILARITY COEFFICIENTS

These are important in constructing ordinations, in which dissimilarities (d) between pairs of samples are turned into distances (d) between sample locations on a "map". (d therefore >0, of course).

Similarities can easily become dissimilarities, by:

$$\delta = 100 - S \qquad (2.11),$$

e.g. for BRAY-CURTIS:

$$\delta_{jk} = 100.\frac{\Sigma_{i=1}^{p} |y_{ij} - y_{ik}|}{\Sigma_{i=1}^{p} (y_{ij} + y_{ik})} \qquad (2.12),$$

so $\delta=0$: no dissimilarity, $\delta=100$: total dissimilarity.

Other dissimilarity measures, based on distances:

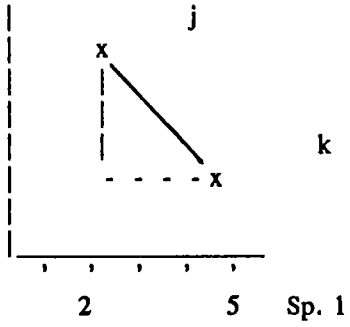
EUCLIDEAN DISTANCE: $d_{jk} = \sqrt{[\Sigma_{i=1}^{p} (y_{ij} - y_{ik})^2]}$ (2.13)

MANHATTAN (or CITY-BLOCK) DISTANCE:

$$d_{jk} = \Sigma_{i=1}^{p} |y_{ij} - y_{ik}| \qquad (2.14)$$

Example:

Sample:	j	k
1	2	5
Sp. 2	3	1



___ Euclidean

- - Manhattan

[METRICS: Euclidean and Manhattan measures, (2.13) and (2.14), are called distances or metrics because they obey the triangle inequality, i.e. for any three samples, j,k,r:

$$d_{jk} + d_{kr} \geq d_{jr} \qquad (2.15).$$

Note: Bray-Curtis dissimilarity does not satisfy the triangle inequality, so should not be called a "metric". However, many useful dissimilarities are also not metrics (e.g. squared Euclidean distance, giving dissimilarities of the same rank order as Euclidean distance, i.e. identical MDS ordinations).

CONCLUDE: Unnecessary to insist that dissimilarities are true "metrics".]

Where necessary (e.g. for input to clustering), distance (d) can be conveniently converted to similarity (S) by:

$$S = 100/(1 + d) \qquad (2.16),$$

and, using (2.11), distance (d) turned to dissimilarity (d) by

$$\delta = 100d/(1 + d) \qquad (2.17).$$

So, $d = 0$ gives $\delta = 0$, $S = 100$, and $d \to \infty$ gives $\delta \to 100$, $S \to 0$.

However, note that EUCLIDEAN (or MANHATTEN) distance is the same if a species is absent in both samples or is present in both at the same abundance; this is undesirable. (Same problem as that of similarities based on correlation being dependent on joint absences.) So:

RECOMMENDATION: For clustering or MDS of species counts/biomass, use Bray-Curtis dissimilarities, after suitable transformation, rather than Euclidean (or Manhatten) distances.

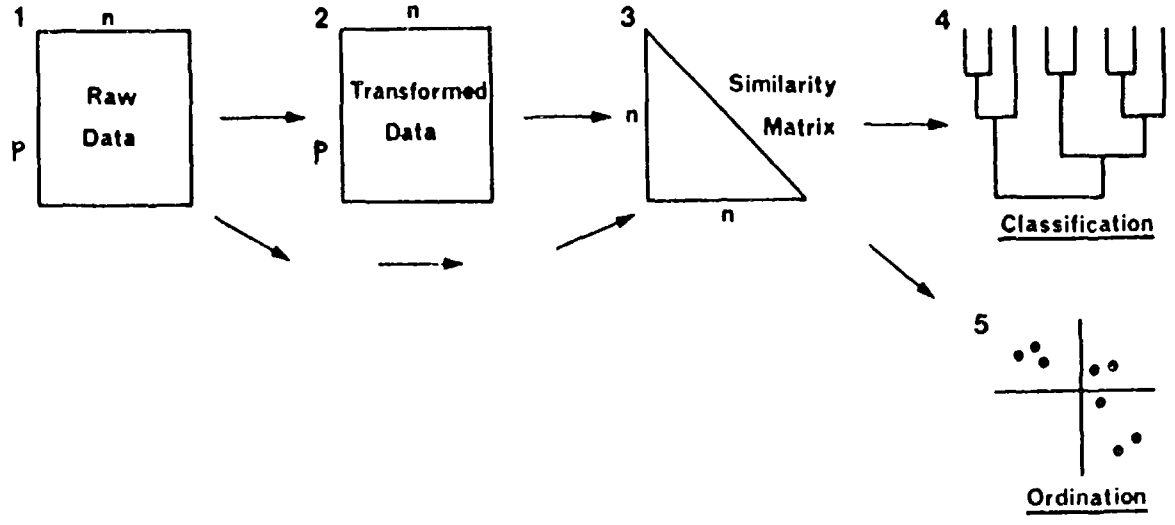


Fig. 2.1 Stages in a multivariate analysis based on (dis)similarity coefficients.

LECTURE 3

MULTIVARIATE METHODS: HIERARCHICAL CLUSTERING

	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
A1	-											
A2	61	-										
A3	69	60	-									
A4	65	61	66	-								
B1	37	28	37	35	-							
B2	42	34	31	32	55	-						
B3	45	39	39	44	66	66	-					
B4	37	29	29	37	59	63	60	-				
C1	35	31	27	25	28	56	40	34	-			
C2	40	34	26	29	48	69	62	56	56	-		
C3	40	31	37	39	59	61	67	53	40	66	-	
C4	36	28	34	37	65	55	69	55	38	64	74	-

Table 3.1. Frierfjord macrofauna counts. Similarities (Bray-Curtis coefficient, after $\sqrt{\sqrt{}}$ transformation) between every pair of replicates (sites A-C only).

Seeing structure in a similarity matrix is difficult - a graphic representation of relations is needed:

CLUSTER ANALYSIS. Clustering (or classification) aims to find "natural groupings" of samples such that samples within a group are more similar than samples in different groups. Use clustering to:

1) Distinguish sites (or times) - replicates within sites fall in the same cluster;

2) Partition sites (or times) into groups;

3) Define species assemblages (spp. co-occur at sites)

Hundreds of clustering methods exist (Everitt 1980), some operating on (dis)similarities, some on raw data. Cormack (1971) warns against indiscriminate use: "availability of ... classification techniques has led to the waste of more valuable scientific time than any other 'statistical' innovation"

Five classes of clustering methods can be defined:

1) Hierarchical,
2) Optimising,
3) Mode seeking,
4) Clumping and
5) Miscellaneous techniques.

Here consider only one (sub)class, which recognises that clustering can occur at several levels.

HIERARCHICAL AGGLOMERATIVE CLUSTERING: The n samples are successively fused into groups, starting with samples with the highest mutual similarites then gradually lowering the similarity level at which groups are fused, and ending in a single cluster. (DIVISIVE clustering is the opposite sequence). Process represented by a tree diagram or DENDROGRAM

DISTINGUISHING SITES: Frierfjord macrofauna counts.

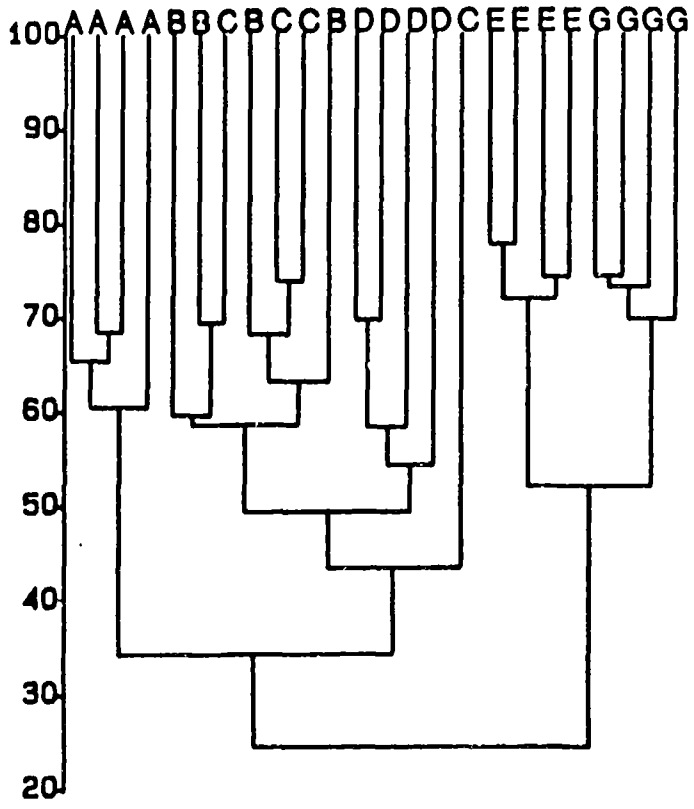


Fig. 3.1. Frierfjord macrofauna counts. Dendrogram for hierarchical clustering (using group-average linking) of 4 replicates from each of sites A-E,G, using Bray-Curtis similarity matrix (Table 3.1).

GROUPING TIMES: Loch Linnhe macrofauna - subset.
After $\sqrt{\sqrt{}}$ transformation, data array and Bray- Curtis similarity matrix are:

Year:	64	68	71	73
Sample:	1	2	3	4
Species				
Echin.	1.7	0	0	0
Myrio.	2.1	0	0	1.3
Labid.	1.7	2.5	0	1.8
Amaea.	0	1.9	3.5	1.7
Capit.	0	3.4	4.3	1.2
Mytil.	0	0	0	0

Sample	1	2	3	4
1	-			
2	25.6	-		
3	0.0	67.9	-	
4	52.2	68.1	42.0	-

↓ 2 & 4 fused

Sample	1	2 & 4	3
1	-		
2 & 4	38.9	-	
3	0.0	55.0	-

↓ (2 & 4) & 3 fused

Sample	1	2 & 3 & 4
1	-	
2 & 3 & 4	25.9	-

Samples 2 and 4 have the highest similarity, S(2, 4), so they form the first group.

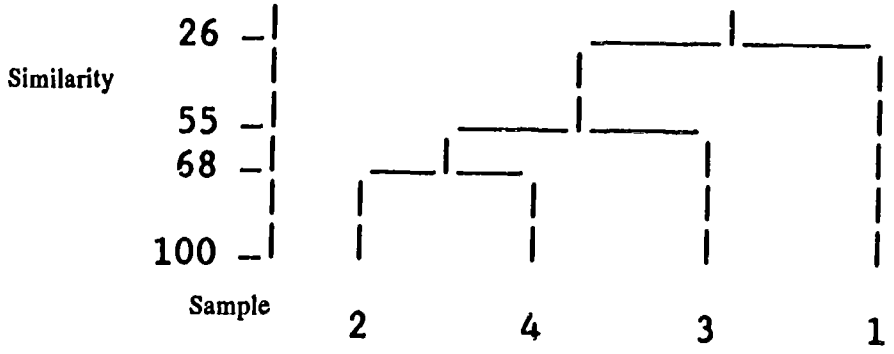
Their similarity to (say) sample 1 defined in one of 3 ways:

a) SINGLE LINKAGE: max{S(1,2), S(1,4)} (= 52.2)

b) COMPLETE LINKAGE: min{S(1,2), S(1,4)} (= 25.6)

c) GROUP AVERAGE LINK: [S(1,2) + S(1,4)]/2 (= 38.9)

(Average weighted by number of samples in groups fused, e.g. S(1,2&3&4) = (2x38.9 + 1x0)/3 = 25.9).



Note:

1) Samples need to be reordered for clear presentation of the dendrogram (so there are no crossing lines).

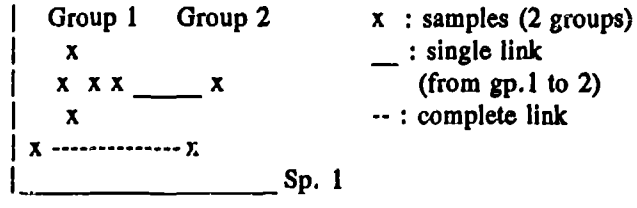
2) The order of samples on the x axis is not very meaningful (think of a dendrogram as a "mobile").

3) Here clustering imposes a (somewhat arbitrary) grouping on what is essentially a continuum (clean (1), impacted (2 and 3) and some recovery (4)), so:

4) small changes in similarities can have larger effects on picture (e.g. reverse S(2,3) & S(2,4)).

DISSIMILARITIES: Exactly converse operations needed for a dissimilarity matrix, i.e. fuse samples with lowest dissimilarity, take minimum dissimilarity in single linkage, maximum in complete linkage.

LINKAGES: These three options are best visualised for an example with only 2 species and dissimilarity defined simply from Euclidean distance.



Group average is mean of all 12 intergroup distances.

Explains why alternative names for the linkages are:

"NEAREST NEIGHBOUR" = single linkage
"FURTHEST NEIGHBOUR" = complete linkage

Note: Though single linkage has some nice theoretical properties (e.g. clustering only a function of rank order of similarities), it has a tendency to give chains of linked samples rather than clear groups; group average linking is usually preferable.

Example: Bristol Channel (UK) zooplankton, April 1974, 57 sites x 24 species, Collins and Williams (1982).

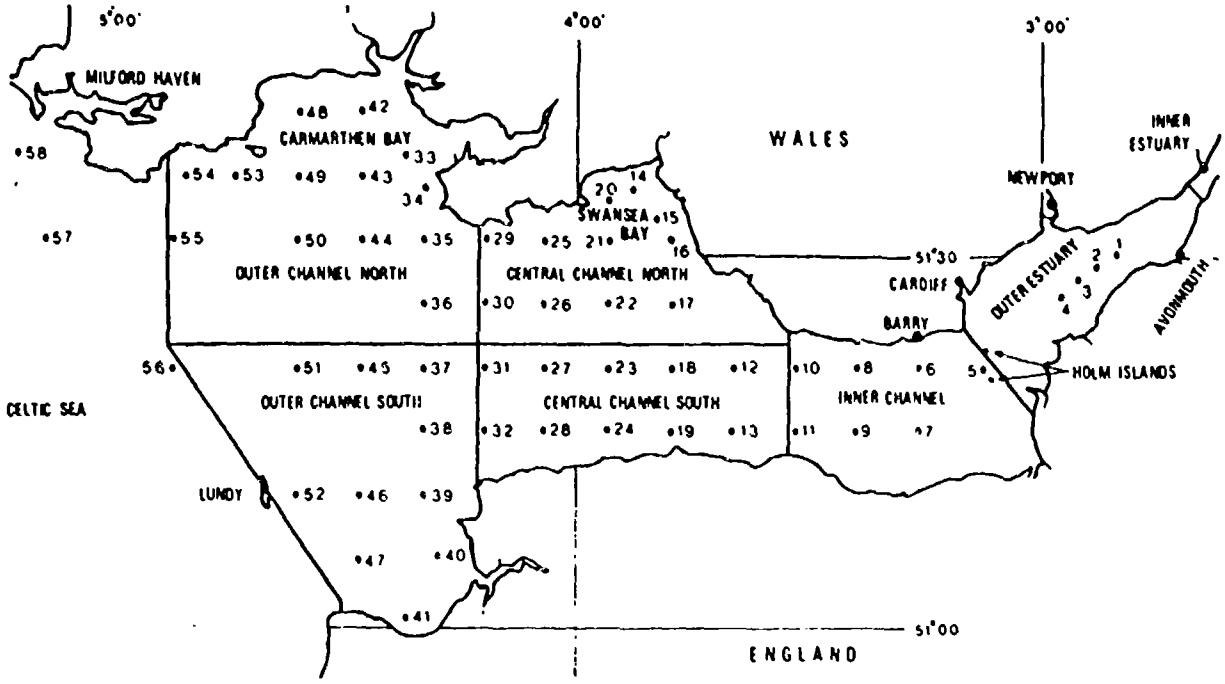


Fig. 3.2. Bristol Channel sampling sites 1-29, 31-58.

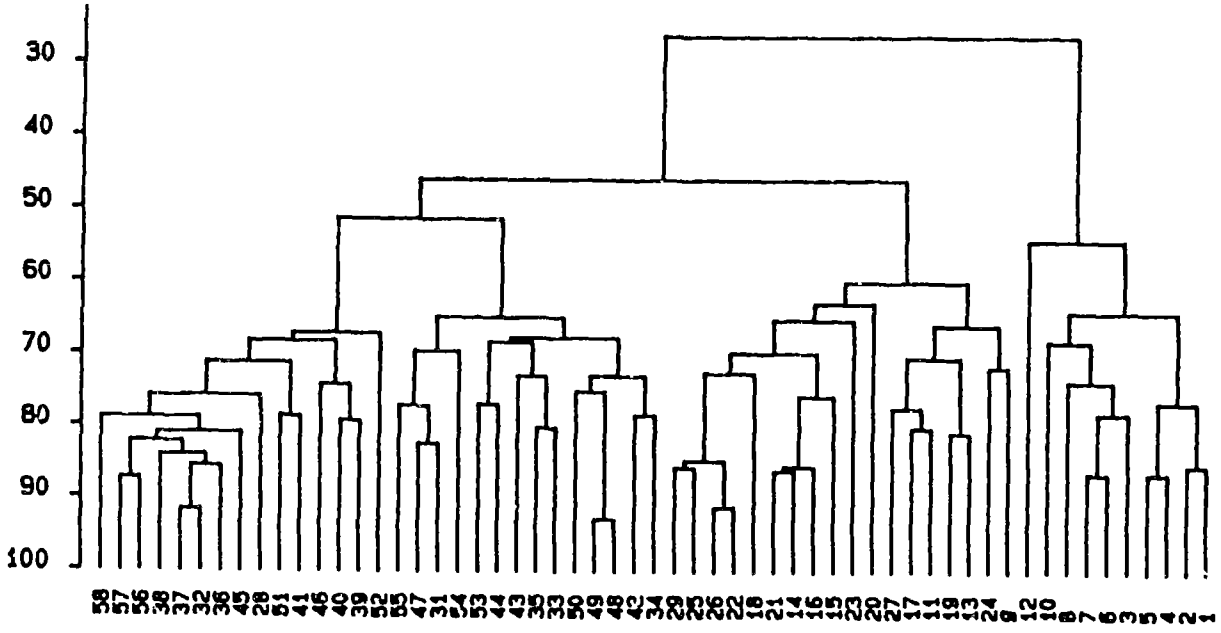


Fig. 3.3. Bristol Channel. Dendrogram for hierarchical clustering of 57 sites (group average linking of Bray-Curtis similarities on $\sqrt{\sqrt{}}$-abundance).

RECOMMENDATIONS

Hierarchical clustering (with group average linking) on sample (dis)similarity matrices can be useful, especially to delineate discrete communities at differing sites (or groups of sites).

It is less useful (and can be misleading) for a gradation in community structure across sites or times; ordination is preferable for this (see lectures 4 and 5).

Clustering is best used in conjuction with an ordination (even for discrete communities), for example, by superimposing clusters on the sample ordination plot.

LECTURE 4

MULTIVARIATE METHODS: ORDINATION OF SAMPLES BY PRINCIPAL COMPONENTS ANALYSIS (PCA)

ORDINATIONS: These are techniques for MAPPING the SAMPLES in a low number of dimensions (usually 2) such that the DISTANCE between samples attempts to reflect (DIS)SIMILARITY in community structure. (No guarantee that the attempt will succeed, if the relationships between the samples are complex, i.e. the structure is essentially "high-dimensional".)

Again there are many methods, for example:

PRINCIPAL COMPONENTS ANALYSIS (PCA, e.g. Chatfield & Collins 1980),
PRINCIPAL CO-ORDINATES ANALYSIS (PCoA, Gower 1966),
DETRENDED CORRESPONDENCE ANALYSIS (DECORANA, Hill & Gauch 1980),
NON-METRIC MULTIDIMENSIONAL SCALING (MDS, e.g. Kruskal & Wish 1978)

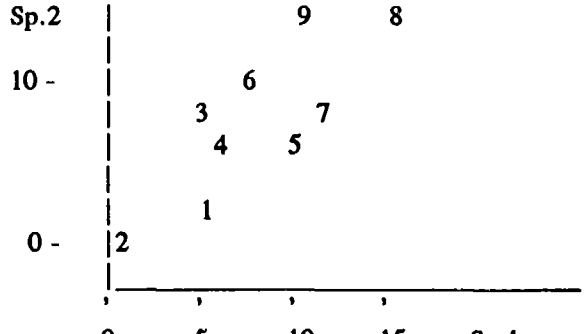
Here we consider only PCA (a simple but rather limited method) and MDS (a more complex algorithm but simple in concept and very generally applicable).

PRINCIPAL COMPONENTS ANALYSIS

STARTING POINT is the original DATA MATRIX (rather than a similarity matrix). The data array is thought of as defining the positions of samples in relation to axes representing the full set of species (one axis for each species). The samples are thus POINTS in a very HIGH-DIMENSIONAL SPACE, so it helps to visualise the process by considering an example in which there are only two species, i.e. each sample is a point in 2-dimensions.

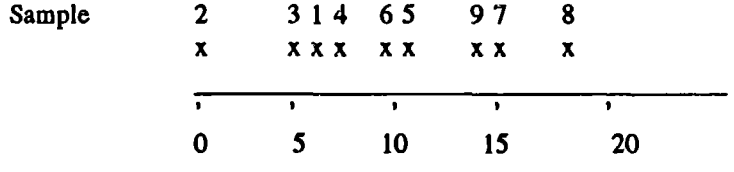
Example: Sample: 1 2 3 4 5 6 7 8 9

Abundance	Sp.1:	6	0	5	7	11	10	15	18	14
	Sp.2:	2	0	8	6	6	10	8	14	14

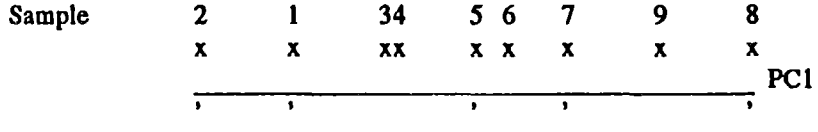


(This is an ORDINATION already - of 2-d data in 2-d, thus perfectly summarising all the relationships between samples.)

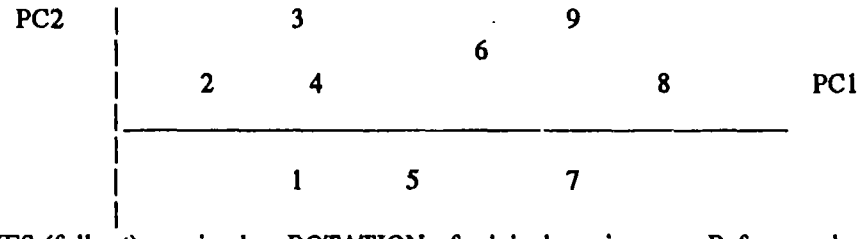
For a 1-d ordination i.e. a genuine ordering of samples) could take just one variable (Sp.1, say):



but this is poorer approximation to relations between samples than given by a (perpendicular) PROJECTION onto the line of "best fit" in the 2-d plot:



This is 1st PC AXIS; PC2 AXIS is PERPENDICULAR to this



PC AXES (full set) are simply a ROTATION of original species axes. Refer samples to (PC1,PC2) rather than (Sp.1,Sp.2) axes because may be able to DISPENSE WITH PC2, giving an ordination in 1-d. Biggest differences between samples take place along PC1, and this is an equivalent definition of PC1 - the axis along which VARIANCE IS MAXIMIZED.

Example: Add a third species to previous example.

	Sample:	1	2	3	4	5	6	7	8	9
Abundance	Sp.1:	6	0	5	7	11	10	15	18	14
	Sp.2:	2	0	8	6	6	10	8	14	14
	Sp.3:	3	1	6	6	9	11	10	16	15

Samples are now points in 3-d and there are 3 PC axes, again a rotation of the 3 species axes, such that:

PC1: Axis which MAXIMIZES VARIANCE of points PROJECTED PERPENDICULARLY onto it.

PC2: Constrained to be perpendicular to PC1, again chosen to maximize variance along this axis.

PC3: Perpendicular to PC1 and PC2.

The new variables (Pcs) are then just LINEAR COMBINATIONS of the old ones (species), such that PC1, PC2, PC3 are UNCORRELATED.

Here, the three Pcs are:

$$\begin{aligned} PC1 &= 0.62 \times Sp.1 + 0.52 \times Sp.2 + 0.58 \times Sp.3 \\ PC2 &= -0.73 \times Sp.1 + 0.65 \times Sp.2 + 0.20 \times Sp.3 \\ PC3 &= 0.28 \times Sp.1 + 0.55 \times Sp.2 - 0.79 \times Sp.3 \end{aligned} \qquad (4.1)$$

Letting var(PCi) = variance of samples on ith PC axis,
var(Sp.i) = variance on ith species axis (i=1,2,3):

$$\Sigma_i \, var(PCi) = \Sigma_i \, var(Sp.i) \qquad (4.2)$$

so % OF (original) VARIANCE EXPLAINED by ith PC is:

$$var(PCi) \; / \; \Sigma_i \, var(PCi) \qquad (4.3).$$

Here PC1 explains 93%, PC2 6% and PC3 1% of variance.
Little variability (information) in PC3. Ignore it, so

PCA ORDINATION: The PC1 and PC2 axes give a 2-d ordination plane (of "best fit" to the sample points) and points are projected perpendicularly onto this from the higher Pcs (just PC3 here). In this case, the 2-d ordination is almost a perfect summary of the 3-d data (the sample points lie near to a plane in the original 3-d species space).

HIGHER-DIMENSIONAL DATA: Typically, there are many more species (say 30+) but the approach is identical. Samples are points in the 30-d (say) species space; the "best-fit" 2-d plane is found and samples projected onto it to get the 2-d PCA ordination. Success is measured by the % of the variability explained by the first 2 of the 30 PCs.

COMPUTATION: Construction of PCs requires derivation of eigenvalues and vectors of a pxp matrix (p = no. of species), e.g. Chatfield and Collins 1980 (note: knowledge of matrix algebra essential). Problems if p is large (compared with no. of samples), so:

EXCLUDE LESS-COMMON SPECIES: These distort ordination badly (even if the matrix operations are possible). E.g. for Loch Linnhe data, the PCA ordination (Fig. 4.1) excludes species making up <3% of total counts at any site, leaving 29 species from 115.

TRANSFORM REMAINING ABUNDANCES (/BIOMASS) before applying PCA, to avoid over-domination by the very common species. E.g. in Loch Linnhe data, Capitella counts go over 4000; Fig. 4.1 uses $\sqrt{\sqrt{}}$ transform.

Example: Loch Linnhe macrofauna (site 34, 1963-1973).

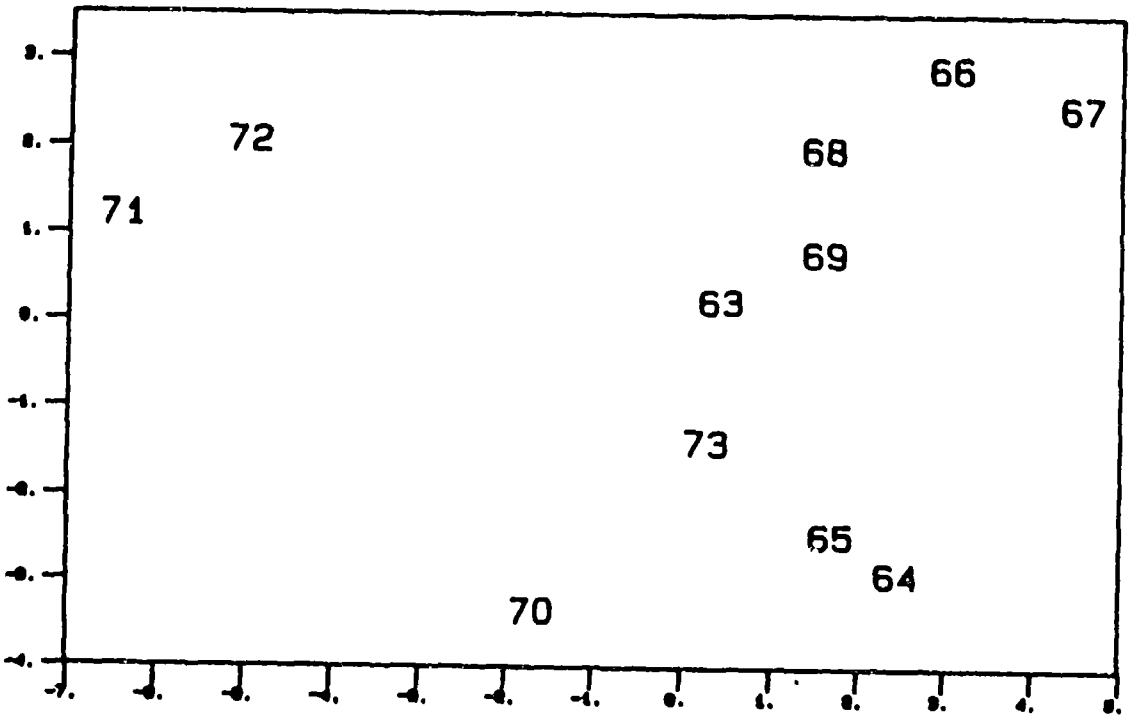


Fig. 4.1. Loch Linnhe abundances. 2-d PCA ordination of samples from 11 years; PC1 (x axis) and PC2 (y axis) account for 57% of total sample variability.

SCALE AND LOCATION CHANGES: Data often NORMALISED (after any transform). For each species subtract the mean (across sites) and divide by the standard deviation. Equivalently, extract eigenvalues of the correlation rather than the covariance matrix, i.e. CORRELATION-BASED PCA rather than COVARIANCE-BASED PCA. Essential if variables have different scales (units) or widely differing ranges. Not the case here (after transform at least) so less necessary (but was done in Fig. 4.1).

PCA STRENGTHS

1) CONCEPTUALLY SIMPLE.

2) COMPUTATIONALLY STRAIGHTFORWARD, provided the number of species is reduced (usually drastically), and it can then cope with an unlimited number of samples.

3) ORDINATION AXES potentially have some meaning, as simple LINEAR COMBINATIONS of the species (though these are rarely readily interpretable in practice).

PCA WEAKNESSES

1) LITTLE FLEXIBILITY in defining relations between samples - in effect "dissimilarities" are simply Euclidean distances in the species space. The only flexibility comes from transformation of the species axes.

2) Does NOT do a very good job of PRESERVING these DISTANCES (dissimilarities) in the 2-d ordination - samples that are far apart in the full space can end up coincident on the 2-d "best-fit" plane, e.g. projected onto it "from opposite sides".

Example: Nematodes from Solbergstrand mesocosm experiment, GEEP Workshop (Warwick et al. 1988).

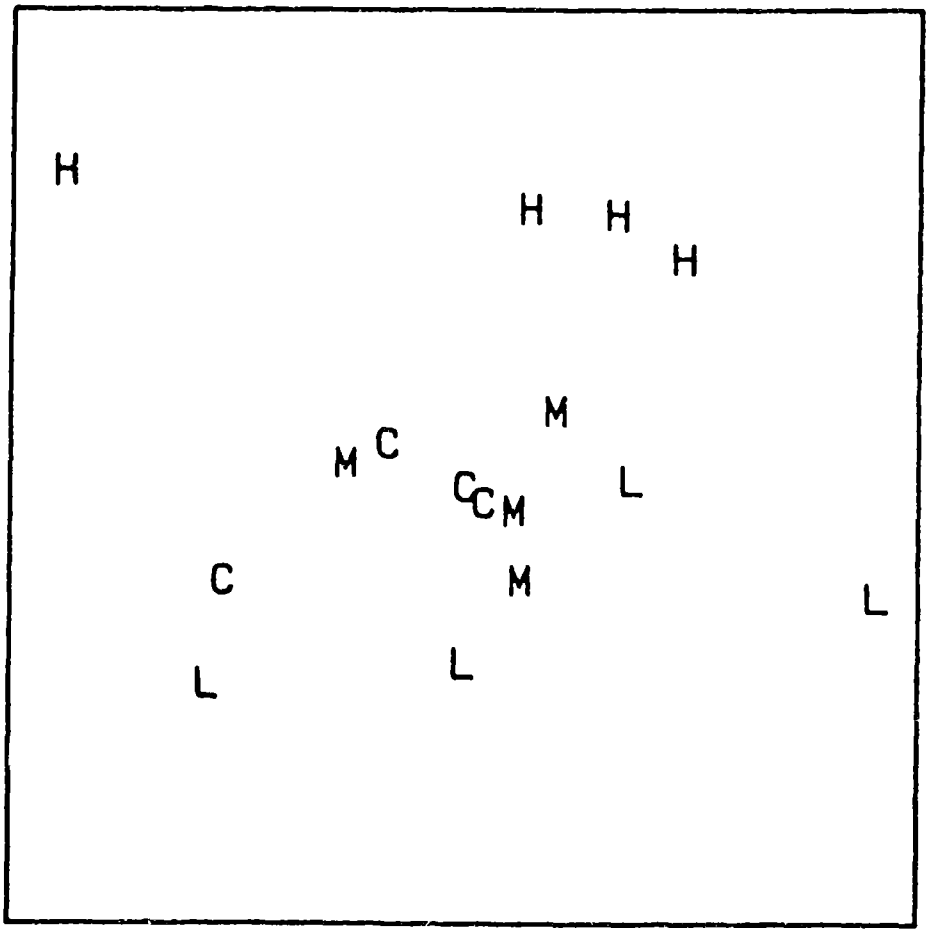


Fig. 4.2. Mesocosm nematodes. Correlation-based PCA of 16 samples: 4 replicate boxes from each of 4 treatments. (C=control, L=low, M=medium and H=high levels of diesel oil and Cu, water dosed for 11 weeks). 26 species retained (usual >3% dominance criterion) - log(1+count) transform applied. PC1 accounts for 23% of variability, PC2 15%.

Strong suggestion of H replicates separating out but note low % of variability explained, so ORDINATION UNRELIABLE. (MDS gives more realistic picture - see Fig 5.5).

LECTURE 5

MULTIVARIATE METHODS: ORDINATION OF SAMPLES BY MULTI-DIMENSIONAL SCALING (MDS)

OTHER ORDINATION METHODS

PRINCIPAL CO-ORDINATES ANALYSIS (PCoA; Gower 1966, Everitt 1978): Also referred to as "CLASSICAL SCALING". Overcomes inflexibility of PCA by allowing WIDER RANGE of DISSIMILARITY definitions; essentially converts these to distances and does a PCA (so still subject to same PCA weakness of poor distance preservation). PCA thus a special case of PCoA, with dissimilarity = Euclidean distance.

DETRENDED CORRESPONDENCE ANALYSIS (DECORANA; Hill and Gauch 1980): Relaxes another constraint of PCA, that of linear combinations of species. Allows CURVILINEAR COMPONENT AXES and can have effect of straightening out "horseshoe" ordinations. But: MDS offers arguably the GREATEST FLEXIBILITY, in the sense of (lack of) assumptions made about the data.

NON-METRIC MULTIDIMENSIONAL SCALING (MDS, e.g. Kruskal and Wish 1978).

STARTING POINT is the (DIS)SIMILARITY MATRIX between samples (i.e. the relevant sample relationships). In fact, the ordination depends only on the RANKS of similarities in the triangular matrix, so is conceptually simple:

MDS attempts to construct a SAMPLE "MAP" (in a given number of dimensions, e.g. 2-d) using information of the form "Sample 1 is closer to Sample 4 (in species composition) than it is to Samples 2 or 3".

Example: Loch Linnhe macrofauna - subset (√√ counts)

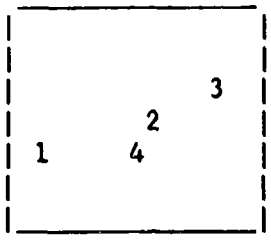
	Year:	64	68	71	73
	Sample:	1	2	3	4
Species					
Echin.	1.7	0	0	0	
Myrio.	2.1	0	0	1.3	
Labid.	1.7	2.5	0	1.8	
Amaea.		0	1.9	3.5	1.7
Capit.		0	3.4	4.3	1.2
Mytil.		0	0	0	0

Similarities

Sample	1	2	3	4
1	-			
2	25.6	-		
3	0.0	67.9	-	
4	52.2	68.1	42.0	-

Rank dissimilarities

Sample	1	2	3	4
1	-			
2	5	-		
3	6	2	-	
4	3	1	4	-



MDS plot

NOTE:

1) MDS plot can be arbitrarily SCALED, LOCATED, ROTATED or INVERTED; it gives positions of samples relative to each other.

2) Not difficult here to place 4 points in 2-d with interpoint distances preserving the rank order dissimilarities exactly. Usually not possible and there will be some distortion or STRESS between (ranked) dissimilarities and corresponding distances in the plot (even in a higher-dimensional ordination).

Example: R. Exe nematodes (Field et al. 1982)

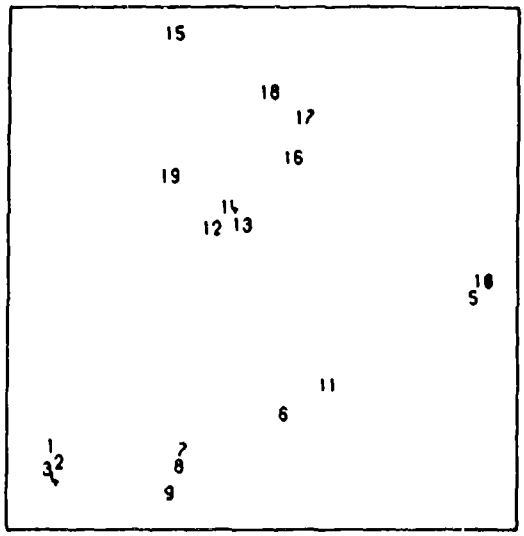


Fig.5.1. Exe nematodes. 2-d MDS ordination of 19 sites, from Bray-Curtis similarities on $\sqrt{\sqrt{}}$ transformed abundances (182 species).

MDS ALGORITHM - an iterative process

1) SPECIFY NUMBER OF DIMENSIONS for MDS plot (= m).

2) CONSTRUCT STARTING "MAP" of n samples; this could be result of (say) a PCA ordination or simply a random set of points (in m-dimensions).

3) REGRESS INTERPOINT DISTANCES $\{d_{jk}\}$ from this map on the corresponding dissimilarities $\{d_{jk}\}$. Can be
   a) LINEAR (or CURVILINEAR) regression -METRIC MDS;
   or, more usually
   b) MONOTONIC (increasing) regression - NON-METRIC MDS (Fig. 5.2).

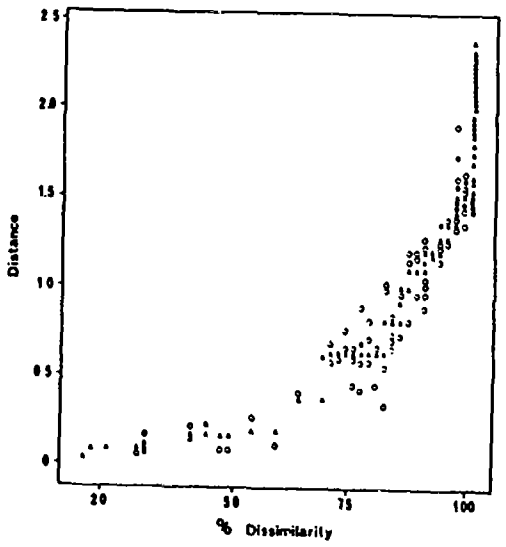


Fig. 5.2. Exe nematodes. "Shepard diagram" of distance (d) in MDS plot (Fig. 5.1) against dissimilarity (δ) in Bray-Curtis matrix.
o = actual distance $\{d_{jk}\}$,
(* = ≥2 coincident points),
▲ = fitted monotonic regression $\{\hat{d}_{jk}\}$.
Stress (= 0.053) is a measure of scatter about the regression line.

4) MEASURE GOODNESS-OF-FIT of the regression by:

$$\text{STRESS} = \Sigma_j\Sigma_k \, (d_{jk} - \hat{d}_{jk})^2 \, / \, \Sigma_j\Sigma_k \, d_{jk}^2 \qquad (5.1)$$

where $\hat{d}_{jk}$ = distance given by the fitted regression line for dissimilarity δ_{jk}.

Stress = 0 if the distances preserve the rank order of the dissimilarities $\{\delta\}$.
Stress is large if the current map is poorly related to the dissimilarities $\{\delta\}$.

5) PERTURB CURRENT SAMPLE POSITIONS on the map, in directions decreasing the stress, using a STEEPEST DESCENT algorithm.

6) REPEAT STEPS 3 TO 5 (regress d on δ, measure stress, perturb points) until no further reduction in stress is possible.

NOTE:

a) The algorithm is an ITERATIVE PROCEDURE so could converge to a LOCAL MINIMUM rather than a global minimum of the stress function.

Also possible to get DEGENERATE SOLUTIONS where most samples collapse to the same point, or to the vertices of a triangle, or are strung out round a circle.

REPEAT FOR DIFFERENT RANDOM STARTING CONFIGURATIONS to confirm that gives same solution (with lowest stress value) several times - this is then very likely the GLOBAL MINIMUM (though not guaranteed).

b) Unlike PCA, a 2-d MDS plot is NOT A PROJECTION of the 3-d plot. Still useful to do the 3-d MDS and use first 2 axes as the start for 2-d MDS - also useful to compare 2-d and 3-d stress values.

<u>ADEQUACY OF MDS REPRESENTATION</u>

1) STRESS VALUE: This increases with increasing number of samples and decreasing dimension of the plot, but roughly speaking, in 2-d:

STRESS
< 0.05 implies excellent representation,
< 0.1 good,
< 0.2 still useful, but
> 0.3 little better than random points.

(An alternative formula with a different denominator, "STRESS2", is preferred by some, but it increases the likelihood of finding local minima and is not recommended for routine use).

2) SHEPARD DIAGRAM: Scatter in this is measured by the stress value (low in Fig. 5.2, stress = 0.053, implying good MDS representation). Diagram also aids detection of "OUTLYING" POINTS and ERRORS in individual dissimilarities.

3) CONNECTION OF SIMILAR SAMPLES: Distortion in an MDS plot seen by connecting points whose similarities are in the top 10% or 20% (say) of values in the similarity matrix.

4) MINIMUM SPANNING TREE (MST): A similar idea - all points in the MDS plot are joined by a SINGLE CONNECTED LINE (which branches but is not allowed to form a closed loop) such that the sum of dissimilarities along this line is minimized; distortion is indicated by connections which look out of keeping with the distances in the plot (see Gower and Ross 1969, for MST algorithm).

5) SUPERIMPOSITION OF GROUPS FROM CLUSTER ANALYSIS: The combination of clustering and ordination can be very effective.

Example: Exe nematodes, 19 sites (182 species)

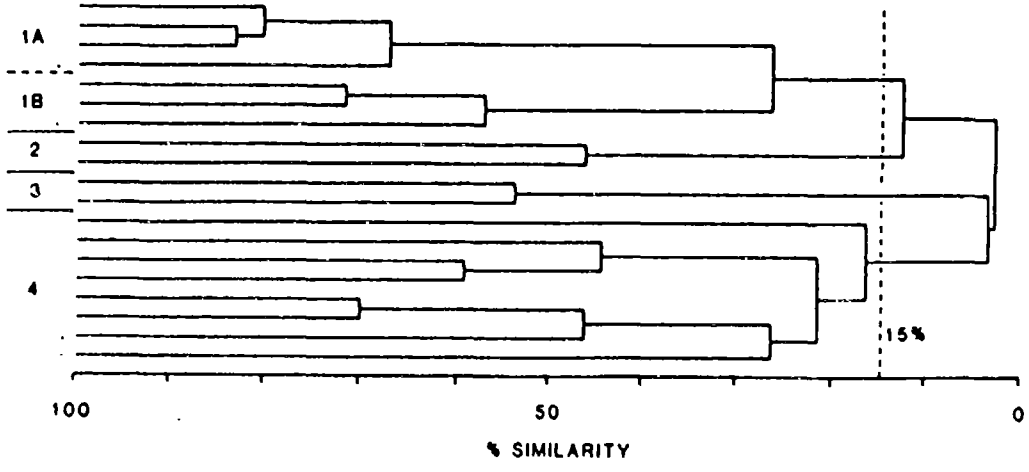


Fig. 5.3. Exe nematodes. Dendrogram (group average linking, Bray-Curtis similarities on √√-abundance). 4 groups of sites separated by 15% similarity cut- off; 8 groups by a 30% (to 45%) threshold.

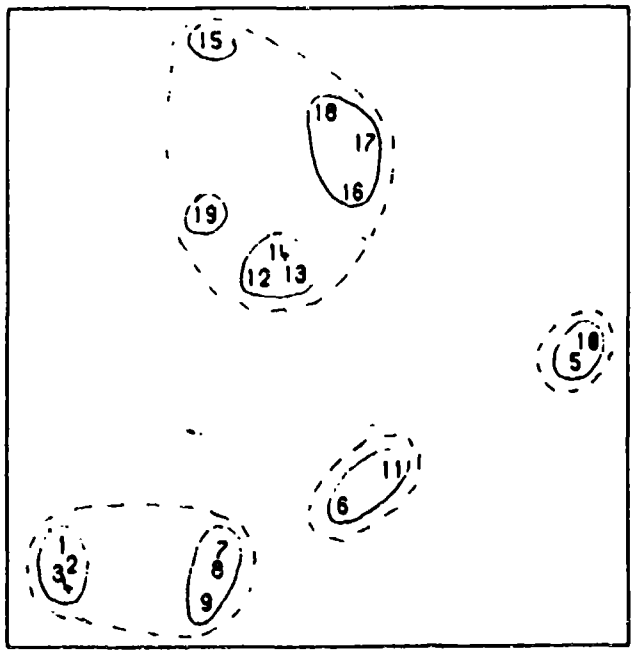


Fig. 5.4. Exe nematodes. MDS (as Fig. 5.1) with clusters indicated at: --- 15%, — 30% similarity.

Agreement clearly excellent (because clusters are sharp and MDS stress low). More revealing example provided by the data of Fig. 4.2:

Example: Mesocosm nematodes, GEEP Workshop.

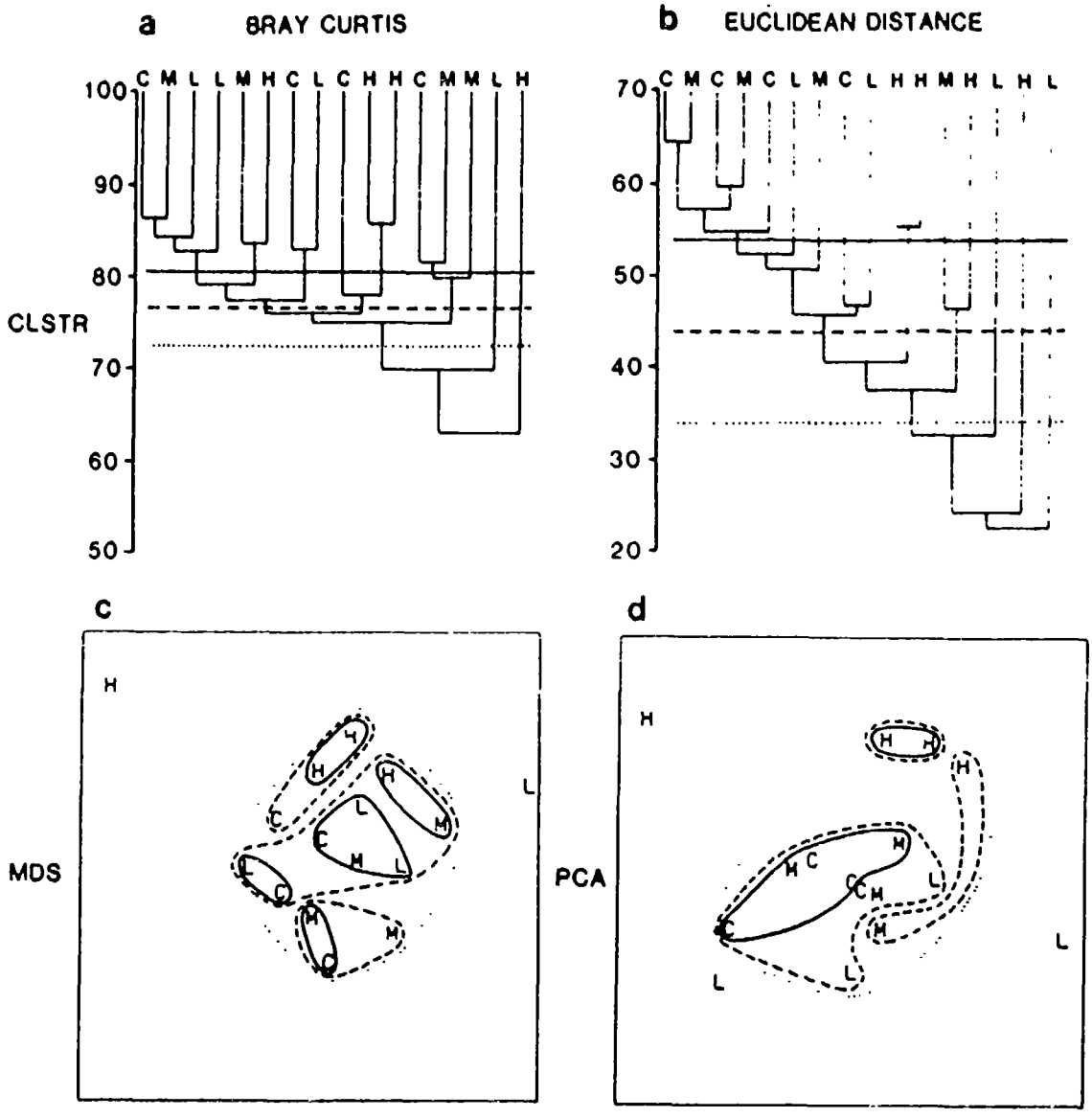


Fig. 5.5. Mesocosm: 4 replicates from 4 treatments (reduced species and log transform, as Fig. 4.2). a),c) Group-average clustering from Bray-Curtis similarities; clusters formed at 3 (arbitrary) levels superimposed on the MDS obtained from the same similarities (stress = 0.19). b),d) Group average clustering from "Euclidean distance" (dis)similarities superimposed on the PCA (Fig. 4.2). (Euclidean distance is the dissimilar- ity measure implicit in a PCA ordination.)

NOTE:

1) Though no natural groupings are apparent from the MDS, the Bray-Curtis cluster and MDS analyses (a and c) are not really inconsistent.

2) The PCA and its corresponding cluster analysis (d and b) are in disagreement, indicating that the 2-d PC axis is a distorted representation of the true "distances" between samples.

ORDINATION v CLUSTERING: Strength of ordination is in displaying GRADATION (rather than categorisation) of community composition in a set of samples.

Example. Celtic Sea zooplankton (Collins, pers. comm.)

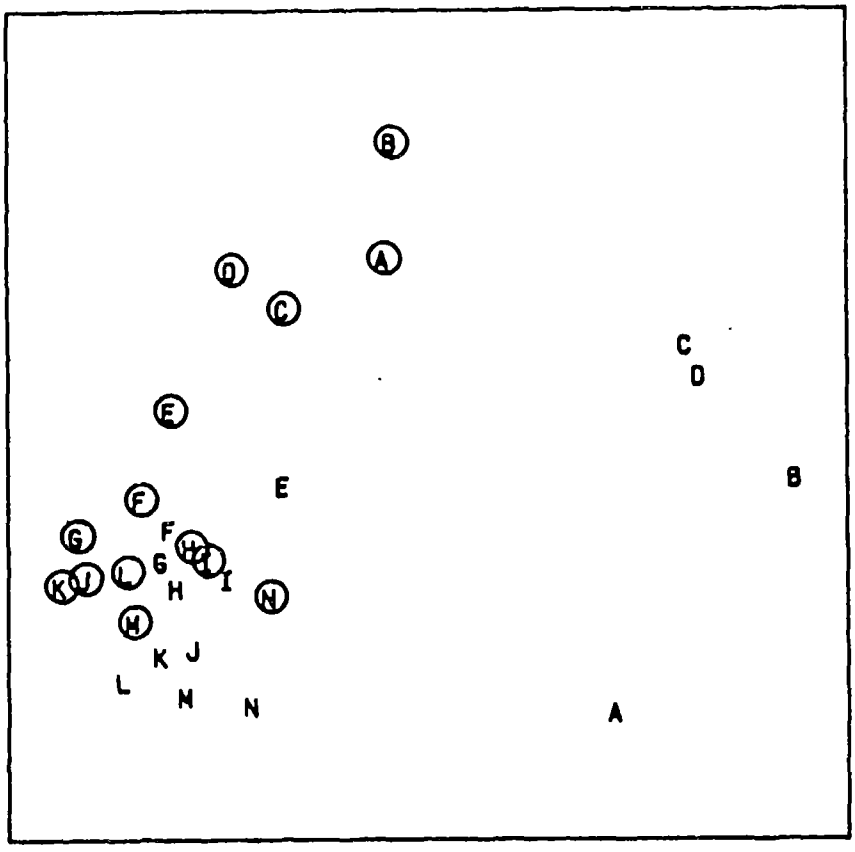


Fig. 5.6. MDS of zooplankton samples at a single site (22/9/78), from 14 depths (5m to 70m, denoted A,B,..,N) for night (circles) and day-time hauls.

MDS STRENGTHS:

1) SIMPLE in concept.

2) BASED ON RELEVANT INFORMATION. It can be used with the most appropriate measure of (dis)similarity for the particular data.

3) SPECIES DELETIONS UNNECESSARY for an ordination of samples (any exclusion dividing line is inevitably arbitrary). The similarity measure can automatically weight rarer species appropriately (and can be chosen to ignore joint absences).

4) GENERALLY APPLICABLE. Since MDS uses only rank order of dissimilarities it makes the weakest possible assumptions about quality of the data.

5) SIMILARITIES CAN BE GIVEN UNEQUAL WEIGHT in constructing the MDS plot (e.g. some samples may be more reliable, perhaps because they are based on combining more replicates).

MDS WEAKNESSES:

1) COMPUTATIONALLY DEMANDING; much more than n = 100 samples is prohibitive (fewer on a PC; CPU time is proportional to n^2).

2) CONVERGENCE to the correct solution (the global minimum of stress) is NOT GUARANTEED, since MDS is an iterative procedure; the necessary repeats add to the computational burden.

3) ALGORITHM PLACES MOST WEIGHT ON LARGE DISTANCES. For detailed structure within large clusters it is sometimes necessary to ordinate clusters separately (same constraint applies to most methods, eg. PCA).

RECOMMENDATIONS:

1) MDS RECOMMENDED as one of the best (perhaps the best) ordination technique (e.g. Everitt 1978, Kenkel and Orloci 1986). Preferable to PCA because of its flexibility and (lack of) assumptions.

2) When sample relationships are simple (e.g. a few strong clusters; one strong gradient) most ordination methods will perform adequately. MDS scores because of its greater ability to REPRESENT MORE COMPLEX RELATIONS in 2-d space.

3) If stress is low (say, <0.1), an MDS ordination is probably a more useful representation than a cluster analysis, even when the samples are strongly grouped. However, the techniques complement each other, so PERFORM BOTH, AND VIEW THEM IN COMBINATION, especially for higher stress. (In the latter case also try a higher-dimensional ordination).

LECTURE 6

MULTIVARIATE METHODS: TESTING FOR DIFFERENCES BETWEEN GROUPS OF SAMPLES

DISTINGUISHING SITES (or TIMES) by formal significance tests is a necessary first step to INTERPRETING differences (e.g. control v. impacted site) but usually overlooked for multivariate methods (because of unavailability of suitable tests).

(Note: Cluster analysis will always find clusters, even from random data points!)

UNIVARIATE TESTS

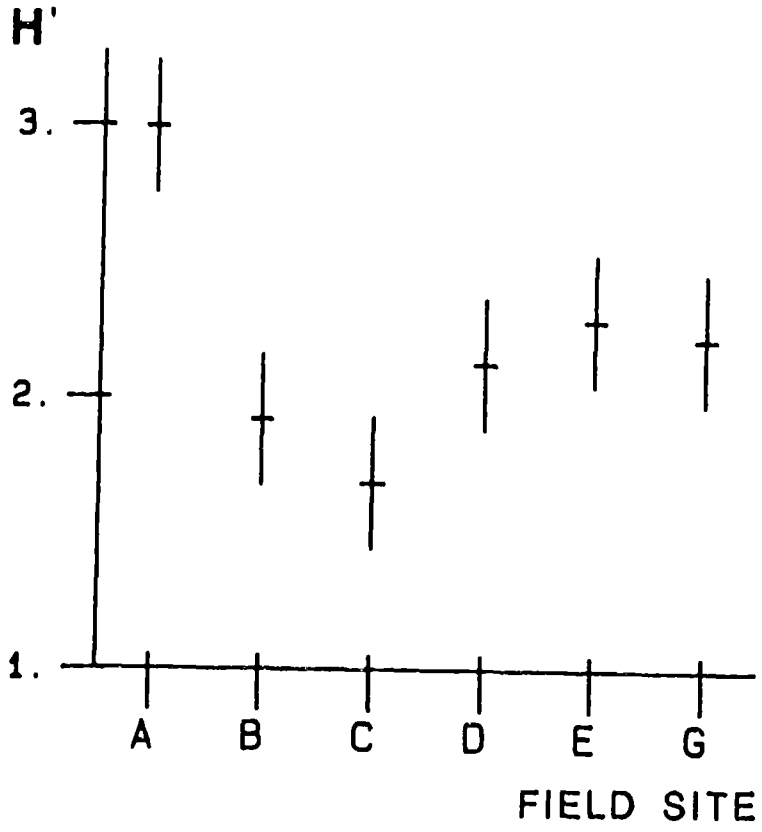


Fig 6.1. Frierfjord macrofauna. Means and 95% confidence intervals for Shannon diversity (H') at 6 field sites.

ONE-WAY ANOVA provides a test of the (null) hypothesis:

H_o: No difference between sites

It assumes normality of H' and constant variance across sites (hence the confidence intervals in Fig. 6.1 use a pooled variance estimate and are of the same widths).

	Sum of squares	Deg. of freedom	Mean Square	F ratio	Sig. level
Treatments	3.938	5	0.788	15.1	<0.1%
Residual	0.937	18	0.052		
Total	4.874	23			

Table 6.1. Frierfjord macrofauna diversity H'; ANOVA.

MULTIPLE COMPARISON TESTS are used to follow up a significant F-test with comparison between (all) pairs of sites, e.g.

TUKEY T TEST (i.e. a Least Significant Difference test) shows that the "reference" site A has significantly higher diversity than the rest, and C has a lower H' than E and G.

NOTE:

1) Multiple comparison tests FIX the PROBABILITY of TYPE I ERROR ("reject the null hypothesis when true") at 0.05 (say) over all pairwise comparisons.

2) Global F-test is best thought of as a "red light" - unless signficant it BARS PROGRESS TO PAIRWISE COMPARISONS and interpretation of differences.

3) There are several implications for SAMPLE COLLECTION, which apply equally to the multivariate testing which follows:

IMPLICATIONS FOR DESIGN

1) CONTROL (reference) site(s) essential - impact only established by reference to similar unimpacted site(s), or to same site pre-impact. (Preferable to have both spatial and temporal controls).

2) REPLICATION at each site essential - should be over appropriate spatial scale (i.e. genuinely representative of that location).

3) "BLIND" ANALYSIS desirable - avoids (unconscious) biases, e.g. tendency to uniformity of replicates.

MULTIVARIATE TESTS

INFORMAL: CLUSTER, MDS, etc. assume no knowledge of how samples are divided into sites. So, plots can be inspected for evidence of REPLICATE GROUPING.

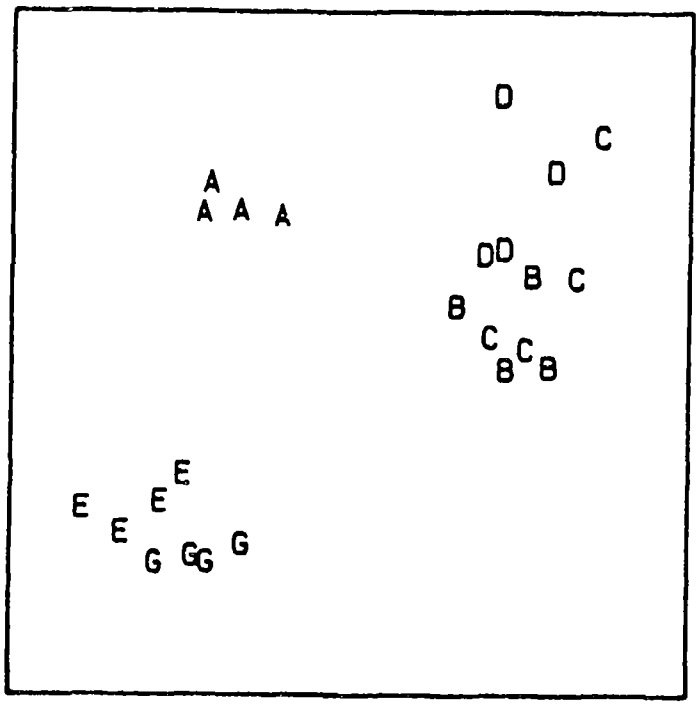


Fig. 6.2. Frierfjord macrofauna. MDS plot (Bray- Curtis similarities, $\sqrt{\sqrt{}}$ transform), for 24 samples, 4 replicates from each of sites A-E,G.

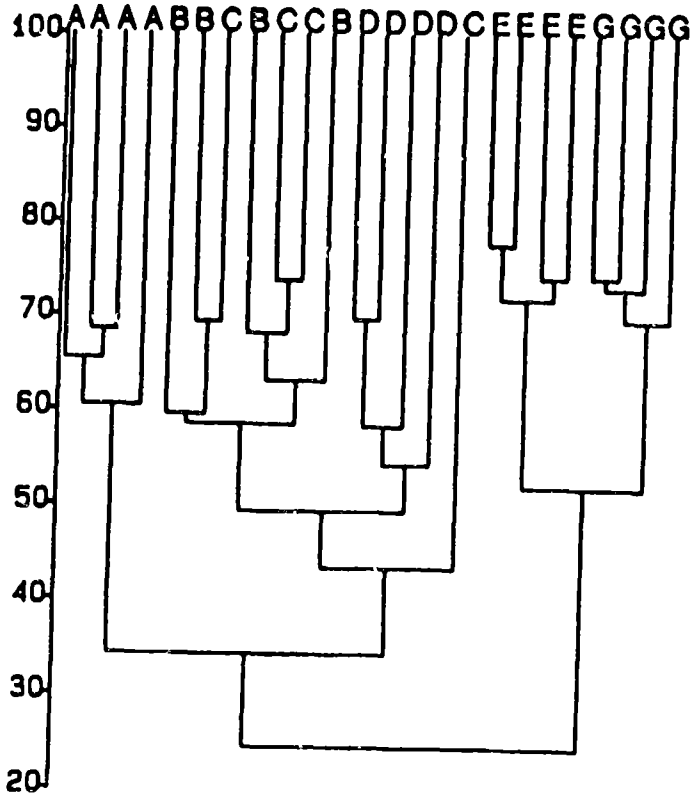


Fig. 6.3. Frierfjord macrofauna. Dendrogram for 24 samples (similarities as for Fig. 6.2).

PARAMETRIC TESTS

EXACT ANALOGUE OF ONE-WAY ANOVA is multivariate analysis of variance (MANOVA), the F-test being replaced by WILKS' K test (e.g. Mardia 1979). Pairwise differences can be tested by MAHALANOBIS' DISTANCES (e.g. Seber 1984); but

ASSUMPTIONS RARELY SATISFIED: Tests require multivariate normality of abundances and "large samples" (at each site). For Frierfjord macrofauna, even after reduction to 30 species:

a) 50% of abundances are zero - normality impossible (even with transform),
b) ratio of observations to parameters needing estimation is 1.1 - hardly large!

RANDOMISATION/PERMUTATION TESTS:

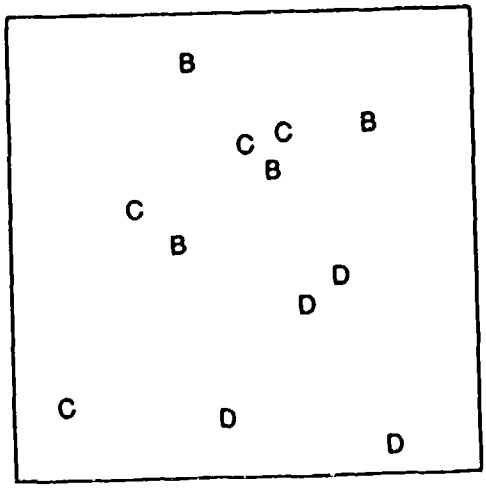


Fig. 6.4. Frierfjord macrofauna. MDS plot (Bray- Curtis, $\sqrt{\sqrt{}}$ transform) of 4 replicates from B,C,D.

NULL HYPOTHESIS H_o: no difference between sites.

If H_o false, distances between replicates within sites are less than distances across sites. So:

1) COMPUTE STATISTIC reflecting this difference. To derive its sampling distribution, note that when H_o true, the 12 labels (4 B's, 4 C's, 4 D's) could be allocated at random to the 12 MDS points. So:

2) RECOMPUTE STATISTIC under ALL POSSIBLE PERMUTATIONS of the 12 labels between the 12 MDS points, or (since that is prohibitive) under a LARGE NUMBER OF RANDOM ALLOCATIONS of the 12 labels to the points.

3) RANDOMIZATION/PERMUTATION TEST will reject H_o AT 5% SIGNIFICANCE LEVEL if observed statistic greater than its value for 95% of the random relabellings.

FORM OF DISPLAY SHOULD BE IRRELEVANT: Desirable that the statistic has exactly the same value whether the representation is:

a) a dendrogram (Fig. 6.3)

b) an MDS for all 6 sites (Fig. 6.2) or just a subset of sites (Fig. 6.4)

c) an MDS in 3-d, say, rather than 2-d.

Bearing in mind that MDS is a function only of rank (dis)similarities, this suggests:

STATISTIC based on DIFFERENCE IN AVERAGE RANK DISSIMILARITIES between and within sites, i.e.

$$R = (\bar{r}_{Between} - \bar{r}_{Within})/(M/2) \qquad (6.1)$$

where $M = n(n-1)/2$ (n = total number of samples) and:

$R = 1$ if all replicates within sites are more similar than any replicates between sites.

$R = 0$ represents the null hypothesis.

($R < 0$ possible, but only significantly so if experimental design incorrectly specified).

PAIRWISE COMPARISONS OF SITES: If global test rejects H_0 then same type of test can be carried out on each pair of sites, though note:

a) These tests must be treated with some caution since NOT true "MULTIPLE COMPARISON" TESTS; overall Type I error not controlled.

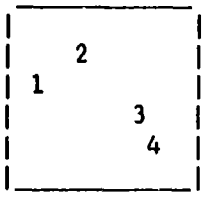
b) Minimum of 4 replicates per site needed for pairwise tests. Can be fewer for global test since NUMBER OF DISTINCT PERMUTATIONS is:

$$(\Sigma_i n_i)!/(n_1!n_2!\ldots n_k!k!) \qquad (6.2)$$

where $\{n_i\}$ replicates at site i ($i=1,2,..,k$).

Example: 2 replicates at each of 2 sites (A,B)

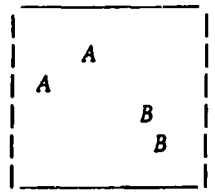
Sample	A 1	A 2	B 3	B 4
A 1	-			
A 2	2	-		
B 3	4	3	-	
B 4	6	5	1	-



Rank dissimilarities *MDS (1,2 = A; 3,4 = B)*

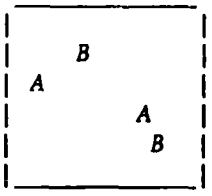
$\bar{r}_{Between} = 4.5$, $\bar{r}_{Within} = 1.5$, M = 6, so R = 1.

Only three possible distinct PERMUTATIONS OF LABELS:



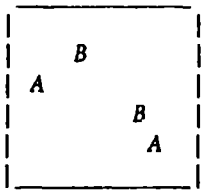
Smp.	A 1	A 2	B 3	B 4
A 1	-			
A 2	2	-		
B 3	4	3	-	
B 4	6	5	1	-

R = 1



Smp.	A 1	A 3	B 2	B 4
A 1	-			
A 3	4	-		
B 2	2	3	-	
B 4	6	1	5	-

R = -0.5



Smp.	A 1	A 4	B 2	B 3
A 1	-			
A 4	6	-		
B 2	2	5	-	
B 3	4	1	3	-

R = -0.5

Observed case (R = 1) has 33% probability of occurring by chance, so could not reject the hypothesis of "no difference between sites" (even though the observed case is the most extreme possible, here)

A more realistic example, where there are 12 samples divided between 3 sites (and thus 12!/(4!4!4!3!) = 5775 possible permutations) is given by Fig. 6.4:

Example: Frierfjord macrofauna abundances.

	B1	B2	B3	B4	C1	C2	C3	C4	D1	D2	D3	D4
B1	-											
B2	33	-										
B3	8	7	-									
B4	22	11	19	-								
C1	66	30	58	65	-							
C2	44	3	15	28	29	-						
C3	23	16	5	38	57	6	-					
C4	9	34	4	32	61	10	1	-				
D1	48	17	42	56	37	55	51	62	-			
D2	14	20	24	39	52	46	35	36	21	-		
D3	59	49	50	64	54	53	63	60	43	41	-	
D4	40	12	18	45	47	27	26	31	25	2	13	-

Table 6.2. Frierfjord macrofauna. Ranked dissimilarity matrix (Bray-Curtis, $\sqrt{\sqrt{}}$ transform) between the 12 replicates from sites B,C,D.

GLOBAL TEST:

$r_{Between}$ = 37.54, r_{Within} = 22.72, M = 66, so R = 0.45.

In 500 random relabellings, none of them gave R>0.45, so H_0 rejected at significance level p<.002 (0.2%).

PAIRWISE TESTS:

For each pair of sites, the corresponding subset of the above triangular matrix is extracted, re-ranked and R computed as above, e.g. for B v C, R = 0.23. This time, R can be re-evaluated for all possible relabellings, giving p<12%, so B & C not significantly different (only 35 distinct permutations, so the maximum attainable significance level is 3%).

However, D does differ from B and C (B v D: R = 0.54, p<3%; C v D: R = 0.57, p<3%).

FURTHER FEATURES AND EXTENSIONS:

1) PERMUTATION TEST CONCEPT dates to Mantel (1967) and general Monte Carlo (randomisation) tests discussed by Hope (1968). Practicals use a FORTRAN program called ANOSIM (Analysis of Similarities).

2) ANOSIM test makes NO ASSUMPTION OF "EQUAL VARIANCE"

Example: Coral communities at South Tikus, Thousand Is., Indonesia (Warwick, Clarke & Suharsono 1990)

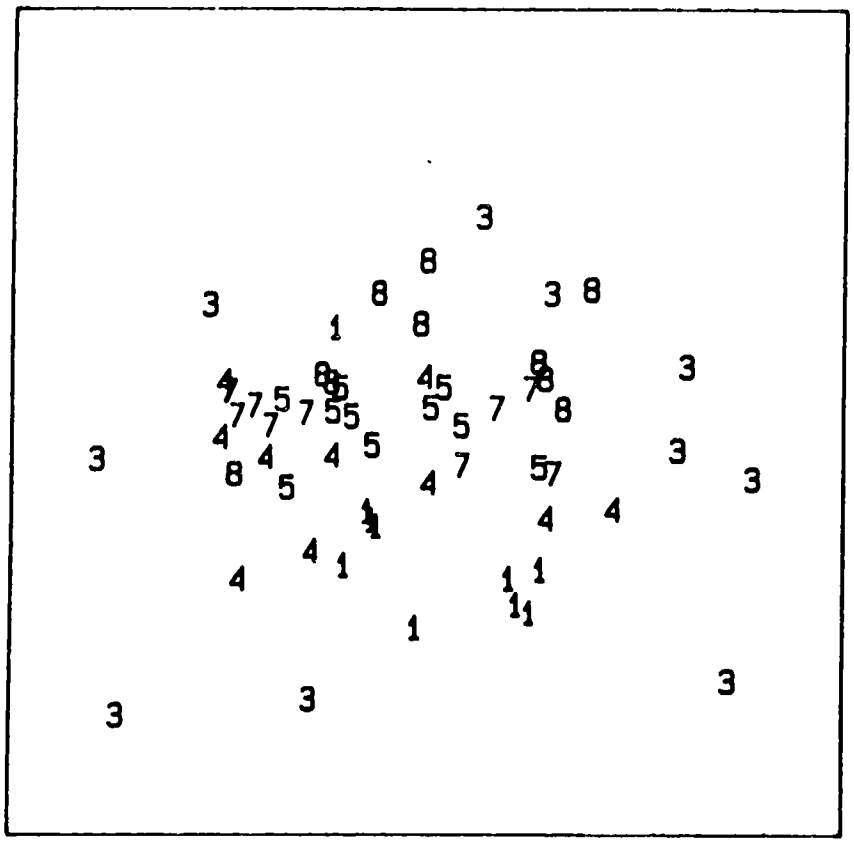


Fig. 6.5. MDS for % cover of coral species (Bray- Curtis, no transform) for 10 replicates in each of 5 years: 1 = 1981 (pre-El Nino), 3 = 1983 etc.

ANOSIM test distinguishes the clear difference in initial and impacted conditions (1 and 3), though change is largely in variance rather than location.

3) ANOSIM TEST NOT RESTRICTED TO BALANCED REPLICATION at sites (or times); some sites can even have only one replicate provided enough replicates overall to generate sufficient permutations (eqt. (6.2)).

4) WIDE APPLICABILITY in that ANOSIM can be used with any (dis)similarity matrix; e.g. for a Euclidean distance matrix (appropriate to a PCA) ANOSIM can be seen as a non-parametric alternative to the parametric Wilks' K test for a MANOVA, though it:

5) LACKS SENSITIVITY (as with many non-parametric tests) in the (unlikely) event that the data is genuinely multivariate normal.

6) ANOSIM PROGRAM EXTENDS TO ANALOGUE OF 2-WAY ANOVA:

2-WAY NESTED MODEL:

Example is Oslo Workshop macrofauna data from the mesocosm experiment: 2 cores from each of 4 boxes from each of 4 treatments.

TEST OF "BOX EFFECTS" involves calculating, separately for each treatment, the 1-way ANOSIM statistic for box differences, and then averaging across treatments. The sampling distribution comes from a restricted randomisation, with permutations preserving treatment designations.

The rank dissimilarity matrix is then reformed for a TEST OF TREATMENT EFFECTS by 1-way ANOSIM.

2-WAY CROSSED MODEL:

Example here would be several sites examined at several times. Can test for any overall differences between times (allowing for site differences by restricting permutations within sites). Alternatively test for overall differences between sites (allowing for differences in times).

RECOMMENDATIONS

1) USE RANDOMISATION/PERMUTATION TEST (ANOSIM) rather than parametric methods for testing of multivariate differences between previously-defined groups of samples (i.e. sites, times, treatments etc.); its ROBUSTNESS (lack of assumptions) more than makes up for its CONSERVATISM - latter is not so bad anyway. (Note: cannot test if differences between groups of samples are 'significant', if the grouping came from multivariate analysis of that same data).

2) USE NORMALITY-BASED TESTS for univariate INDICES, after any necessary transform (see lecture 9).

LECTURE 7

MULTIVARIATE METHODS: SPECIES ANALYSES

SPECIES CLUSTERING

Clustering methods can be applied to SPECIES SIMILARITY matrices (latter defined on p2-5).

Example: R. Exe (UK) nematodes, Field et al. (1982)

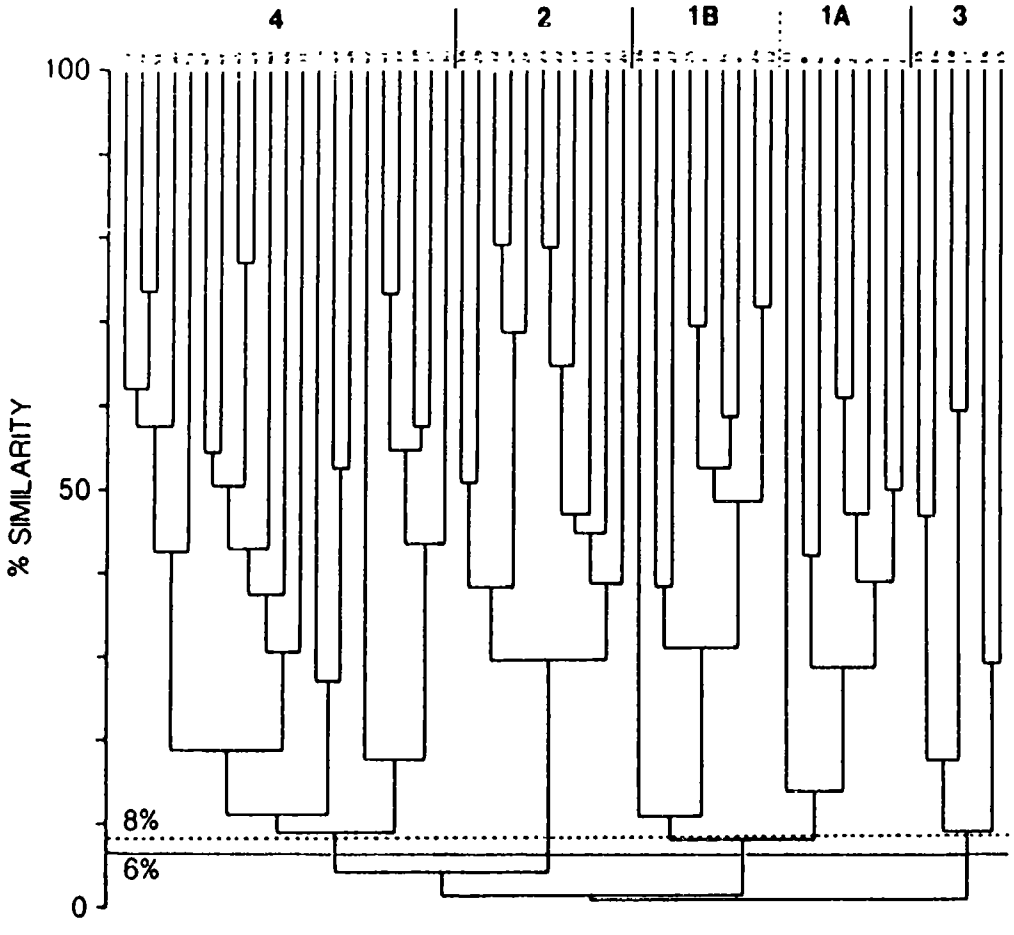


Fig. 7.1. Exe nematodes. Dendrogram (group average link) from Bray-Curtis similarities (standardised abundance data) for 55 species from 19 sites - reduced from 182 species by including those with counts >4% of total at any one site. The 4 to 5 groups indicated correspond closely with sharply defined clusters in the sites analysis (Fig. 5.3).

SPECIES MDS

A species similarity matrix can also be input to an MDS, in the same way as for samples. In practice, often gives high 2-d stress. As with clustering, works best when samples form strong groups, arising from species sets which tend to be exclusive.

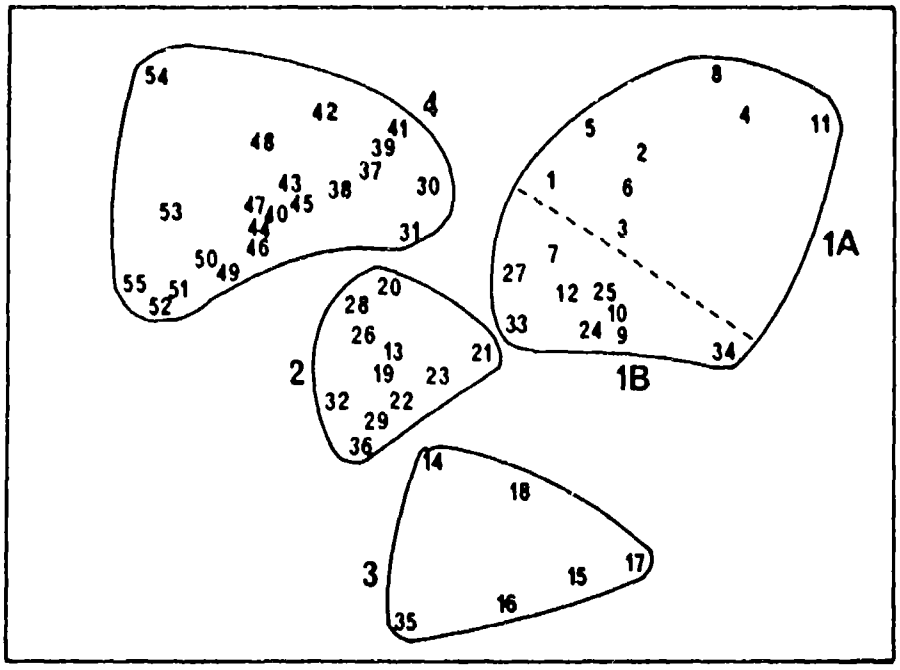


Fig. 7.2. Exe nematodes. MDS of 55 commonest species using Bray-Curtis similarities on standardised abundances. Main groups from cluster analysis (Fig. 7.1) indicated; they correspond closely to groupings of sites (Fig. 5.4).

Note: The LESS-COMMON SPECIES will generate erratic similarities, giving isolated MDS points and an unhelpful plot - they need to be REMOVED initially.

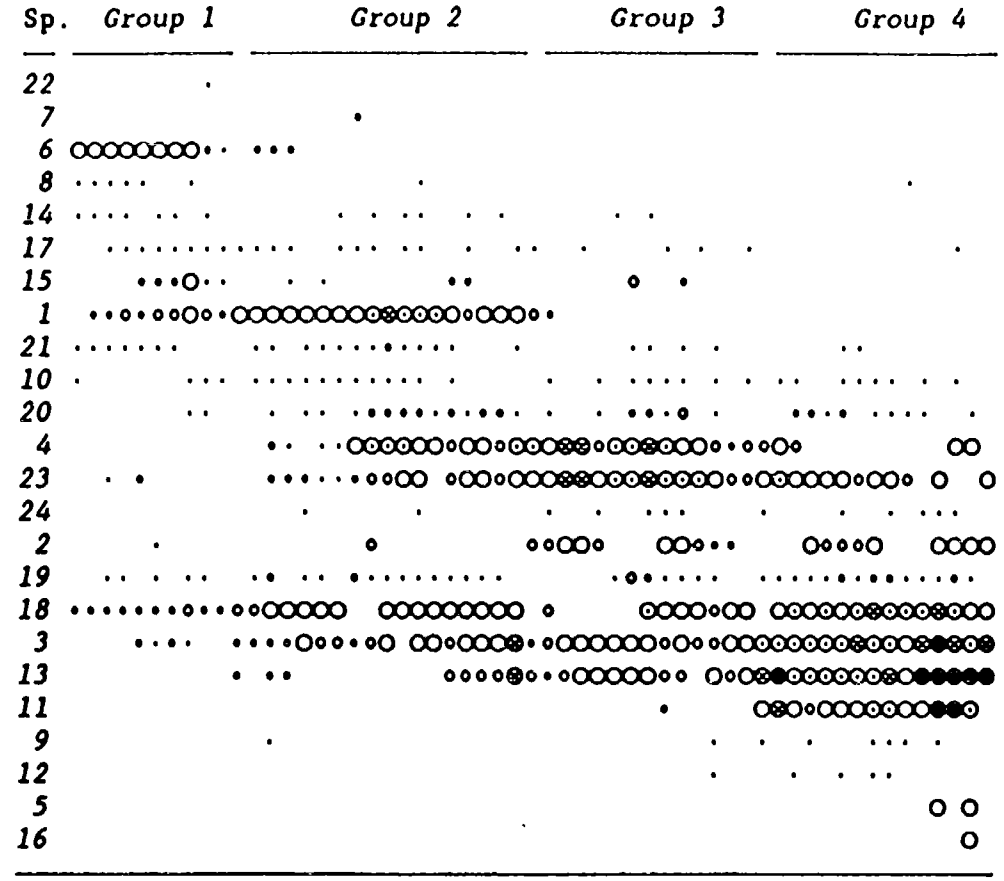
However, SPECIES clustering or ordination is generally less informative than methods which HIGHLIGHT SPECIES contributing to pattern of SAMPLE clustering or ordination:

DETERMINING DISCRIMINATING SPECIES

Given clear CLUSTERING of SAMPLES, what methods will determine SPECIES RESPONSIBLE for groupings? Hard to see patterns in the original data matrix, so:

RE-ORDER COLUMNS (samples) and ROWS (species) to match groupings from site and species clustering and MDS. CATEGORISE counts/biomass and represent by symbols of increasing size & density, to give SHADE MATRIX.

Example: Bristol Channel zooplankton, April 1978.



Group 1: 1,2,4,5,3,6,7,8,10,12
Group 2: 9,24,13,19,27,17,11,20,15,16,14,21,18,25,29,22,26,23
Group 3: 42,34,48,49,50,53,44,43,33,35,54,55,47,31
Group 4: 51,41,45,37,32,36,38,57,56,58,28,39,40,46,52

Fig. 7.3. SHADE matrix for 24 species x 57 sites. Site groups determined by clustering of Fig. 3.3; symbols denote increasing ($\sqrt{\sqrt{}}$-transformed) counts.

Alternative is to BREAK DOWN average DISSIMILARITY ($\bar{\delta}$) between two groups of samples into CONTRIBUTIONS from each SPECIES - revealing GOOD DISCRIMINATORS.

From (2.11), contribution to δ_{jk} from ith species is:

$$\delta_{jk}(i) = 100.|y_{ij} - y_{ik}| \; / \; \Sigma_{i=1}^{p} (y_{ij} + y_{ik}) \qquad (7.1).$$

$\delta_{jk}(i)$ then averaged over all pairs (with j in 1st and k in 2nd group), to give AVERAGE CONTRIBUTION $\bar{\delta}_i$ from ith species (& its standard deviation $SD(\delta_i)$).

DISCRIMINATING SPECIES are those with HIGH $\bar{\delta}_i$ and HIGH ratio $\bar{\delta}_i/SD(\delta_i)$ (this implies CONSISTENCY of contributions across all jk pairs).

Sp.	Name	$\bar{\delta}_i$	SD(δ_i)	$\bar{\delta}_i$/SD(δ_i)	$\Sigma\bar{\delta}_i$%
6	Eurytemora affinis	7.7	2.8	2.7 *	13.0
4	Centropages hamatus	7.3	4.4	1.7 *	25.2
3	Calanus helgolandicus	6.8	4.0	1.7 *	36.7
1	Acartia bifilosa	5.7	4.0	1.4 *	46.3
23	Temora longicornis	5.6	3.3	1.7 *	55.6
18	Pseudocalanus elongatus	4.7	1.5	3.1 *	63.5
13	Paracalanus parvus	3.3	4.2	0.8	69.1
15	Pleurobrachia pileus jv	3.1	2.8	1.1	74.3
20	Sagitta elegans jv	2.9	1.9	1.6 *	79.1
19	Sagitta elegans	2.1	1.6	1.3	82.5
8	Gastrosaccus spinifer	2.0	1.8	1.1	85.9
14	Pleurobrachia pileus	1.9	1.6	1.2	89.0
10	Mesopodopsis slabberi	1.7	1.4	1.3	91.9
21	Schistomysis spiritus	1.6	1.4	1.1	94.5
17	Polychaete larvae	1.5	1.3	1.2	97.1
2	Acartia clausi	0.7	1.8	0.4	98.3
.	.....	...	...	...	....

Table 7.1. *Bristol Channel zooplankton (√√ counts). Species contributions $\bar{\delta}_i$ to total average dissimilarity ($\bar{\delta}$ = $\Sigma\bar{\delta}_i$ = 59.5) between site groups 1 & 2; $\Sigma\bar{\delta}_i$% is cumulative % contribution to $\bar{\delta}$. * denotes good discriminators of groups 1 & 2.*

Can similarly compute the contribution of the ith species ($\bar{S}_i$) to the AVERAGE SIMILARITY WITHIN A GROUP ($\bar{S}$), using the 2nd form of (2.1). This highlights species consistently prominent in that group (i.e. HIGH $\bar{S}_i$, HIGH ratio $\bar{S}_i$/SD(S_i)).

Sp.	Name	$\bar{S}_i$	SD(S_i)	$\bar{S}_i$/SD(S_i)	$\Sigma\bar{S}_i$%
6	Eurytemora affinis	19.3	6.3	3.1 *	29.1
18	Pseudocalanus elongatus	14.7	2.7	5.4 *	51.3
1	Acartia bifilosa	12.2	6.4	1.9 *	69.6
17	Polychaete larvae	3.9	3.1	1.2	75.5
14	Pleurobrachia pileus	3.4	3.8	0.9	80.7
21	Schistomysis spiritus	3.3	3.6	0.9	85.7
15	Pleurobrachia pileus jv	3.3	4.7	0.7	90.7
.	.....	...	...	...	....

Table 7.2. *Zooplankton. Species contribution ($\bar{S}_i$) to average similarity ($\bar{S}$ = 66.3) within site group 1.*

RECOMMENDATION

USE SIMILARITY % BREAKDOWN (programme SIMPER) or a SHADE MATRIX to INDICATE (not test) which species are mainly responsible for an observed clustering of the samples into groups (or for a confirmed difference between previously-defined groups).

LECTURE 8

UNIVARIATE AND DISTRIBUTIONAL METHODS: DIVERSITY MEASURES, DOMINANCE CURVES AND OTHER GRAPHICAL ANALYSES

INDICES OF DIVERSITY AND EVENNESS

A single index of species (or higher taxon) diversity is commonly employed in community studies, and is amenable to simple statistical analysis. A bewildering variety of diversity indices has been used, and it is not appropriate here to discuss their relative merits and disadvantages. A good account can be found in Heip et al. (1988).

Two different aspects contribute to the concept of community diversity:

SPECIES RICHNESS - A measure related to the total number of species present.
EQUITABILITY - Expresses how evenly the individuals are distributed among different species.

The most commonly used diversity measure is the SHANNON-WIENER INDEX:

$$H' = - \Sigma_i \, p_i (\log p_i)$$

This incorporates both the species richness and equitability components. Note that logarithms to the base 2 are often used in the calculation, giving the diversity units as 'bits per individual'. Log_e is also frequently used, so care should be exercised when comparing published indices.

SPECIES RICHNESS is often given simply as the total number of species (S), which is obviously very dependent on sample size, but more commonly as MARGALEF'S INDEX d, which also incoporates the total number of individuals (N):

$$d = (S-1) / \log N$$

EQUITABILITY is most commonly expressed as PIELOU'S EVENNESS INDEX:

$$J' = H'(\text{observed}) / H'_{max}$$

where H'_{max} is the maximum possible diversity (log S).

UNITS OF MEASUREMENT

Numbers of individuals belonging to each species are the most common units. For internal comparative purposes other units could be used, e.g biomass or total cover of each species along a transect (e.g. for hard-bottom epifauna)

REPRESENTING COMMUNITIES

Data usually presented as plots of means and confidence intervals for each site or time.

Example 1: Benthos from Hamilton Harbour, Bermuda.

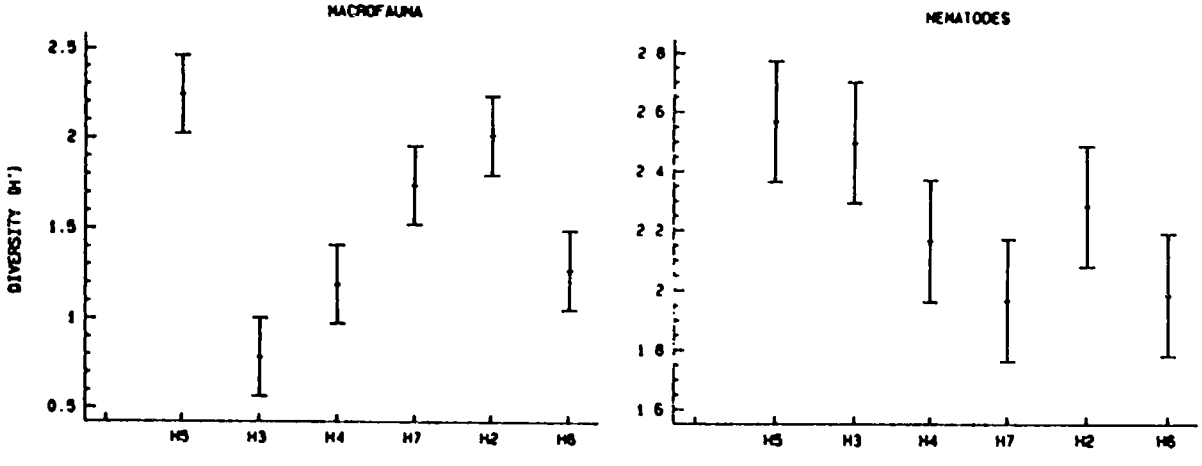


Fig. 8.1. Diversity (H') and 95% confidence intervals for macrobenthos (left) and meiobenthic nematodes (right) at six stations.

Example 2: Reef-corals from South Tikus Island, Indonesia.

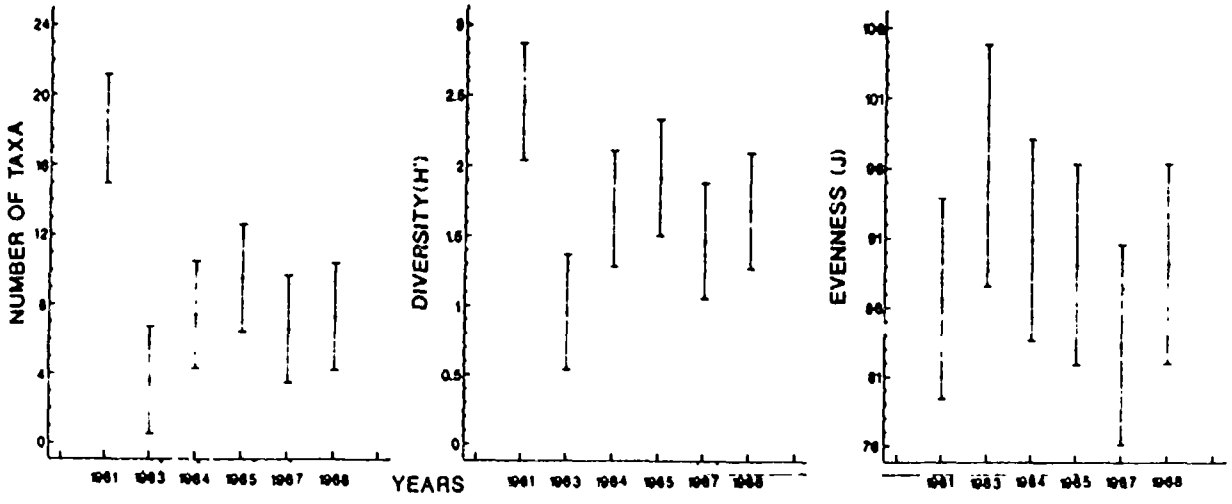


Fig. 8.2. Total number of species (S), Diversity (H') and Evenness (J') based on coral species cover data along transects, spanning the 1982-3 El Niño. Note dramatic decline and partial recovery of S and H', but no obvious changes in J'.

DISCRIMINATING SITES OR TIMES

The significance of differences in diversity indices between sampling sites or times can be tested by one-way analysis of variance (ANOVA).

DETERMINING STRESS LEVELS

Increasing levels of environmental stress are generally considered to:

- DECREASE diversity (e.g. H')
- DECREASE species richness (e.g. d)
- DECREASE evenness (e.g. J'), i.e. INCREASE dominance

Comparisons of measured indices can be made:

- with reference to comparative stations along a spatial contamination gradient (e.g. Fig. 8.1)
- with reference to comparative historical data (e.g. Fig. 8.2)
- with reference to some theoretical expectation of diversity, given the number of individuals and species present. Comparisons of observed diversity have been compared with predictions from CASWELL'S NEUTRAL MODEL (Caswell, 1976), which assumes certain community assembly rules and no interactions between species. A value of zero for the V statistic indicates neutrality, positive values indicate greater diversity than predicted and negative values lower diversity. Values $> +2$ or < -2 indicate significant departures from neutrality. The computer program of Goldman & Lambshead (1989) is useful.

Example: V statistics for summed replicates of macrobenthos and meiobenthic nematode samples at six stations in Hamilton Harbour, Bermuda (cf. Fig. 8.1)

STATION	MACROBENTHOS	NEMATODES
H6	-1.3	-0.4
H2	+0.5	-0.1
H7	-0.2	-0.4
H4	-4.5	-0.5
H3	-5.4	+0.4
H5	-1.9	0.0

Note diversity of macrobenthos at H4 and H3 is significantly below neutral model predictions, but nematodes are close to neutrality at all stations.

GRAPHICAL DISTRIBUTION PLOTS

The purpose of graphical/distributional representations is to extract information on patterns of relative species abundances without reducing that information to a single summary statistic, such as a diversity index. This class of techniques can be thought of as intermediate between univariate summaries and full multivariate analyses. Unlike multivariate methods, these distributions may extract universal features of community structure which are not a function of the specific taxa present, and may therefore be related to levels of biological 'stress'.

RAREFACTION CURVES

Rarefaction curves (Sanders, 1968) were among the earliest to be used in marine studies. They are plots of the number of individuals on the x-axis against the number of species on the y-axis. The more diverse the community is, the steeper and more elevated is the rarefaction curve.

Example: Polychaete/bivalve fraction of macrobenthos.

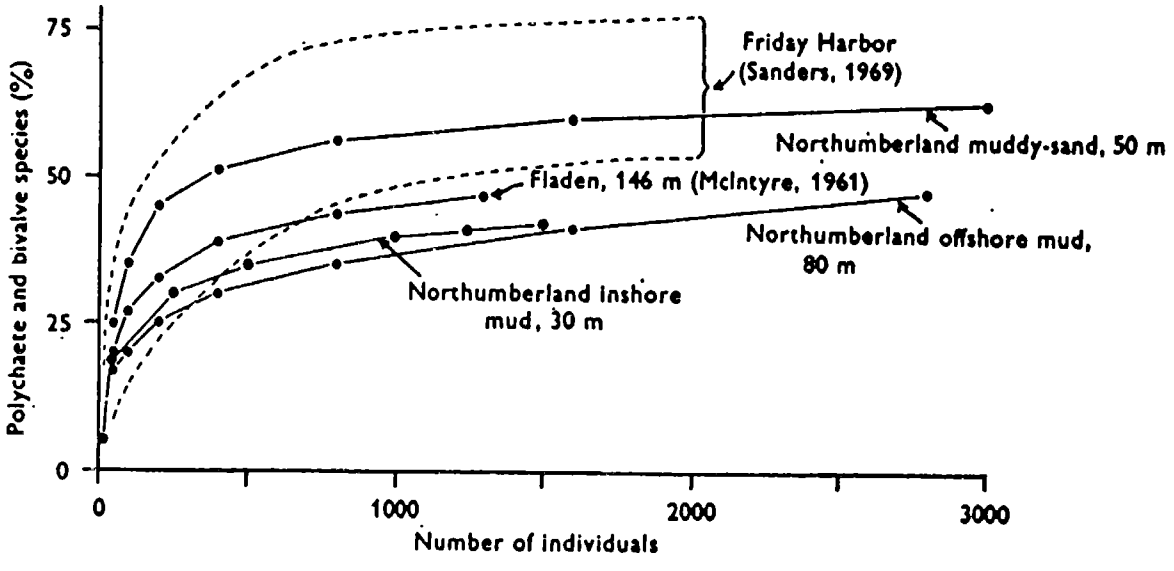


Fig. 8.3. Rarefaction curves comparing North Sea and Friday Harbor stations (from Buchanan & Warwick, 1974)

RANKED SPECIES ABUNDANCE (DOMINANCE) CURVES

These are based on the ranking of species (or higher taxa) in decreasing order of their importance in terms of abundance or biomass. The ranked abundances, expressed as a percentage of the total abundance of all species, are plotted against the relevant species rank. Log transformations of one or both axes have frequently been used to emphasise or downweight different sections of the curves.

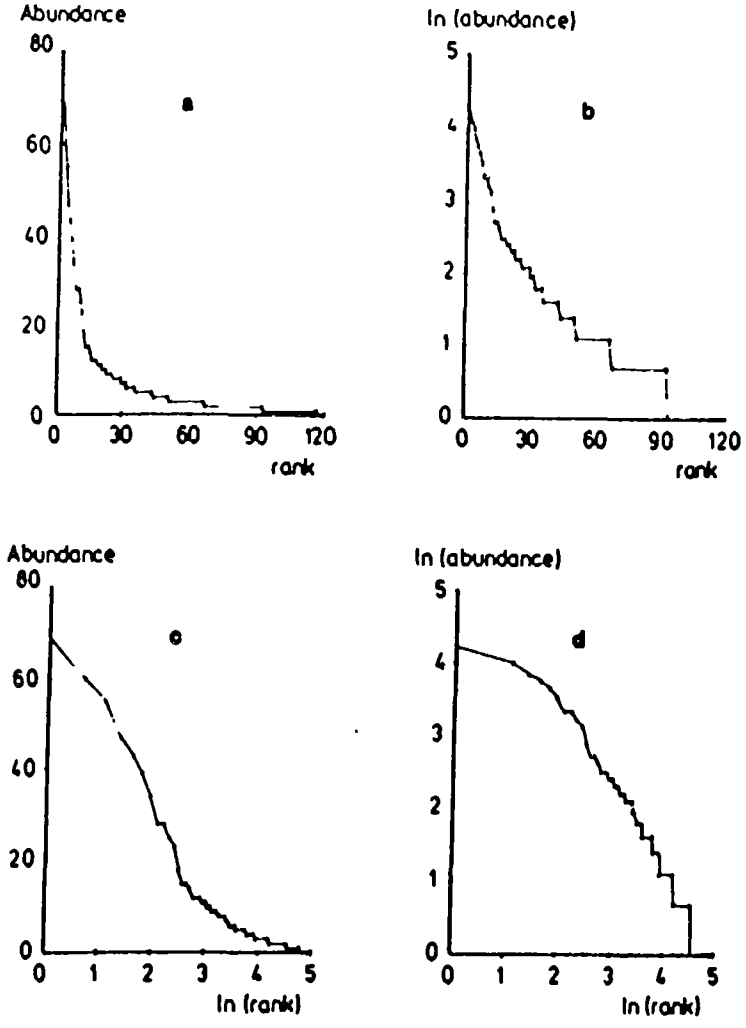


Fig. 8.4. The same (hypothetical) species abundance data plotted as ranked species abundance curves with none, one or both axes on a log scale (from Heip et al., 1988).

k-DOMINANCE AND LORENZ CURVES

As an alternative to the 'simple dominance curves' above, cumulative ranked abundances may be plotted against species rank, or log species rank, to produce k-DOMINANCE CURVES (Lambshead et al., 1983). This has a smoothing effect on the curves. Ordering of curves on a plot will obviously be the reverse of rarefaction curves, with the most elevated curve having the lowest diversity. To compare dominance separately from the number of species, the x-axis (species rank) can be rescaled from 0-100 (relative species rank), to produce LORENZ CURVES.

Example: Nematodes from Loch Ewe, Scotland.

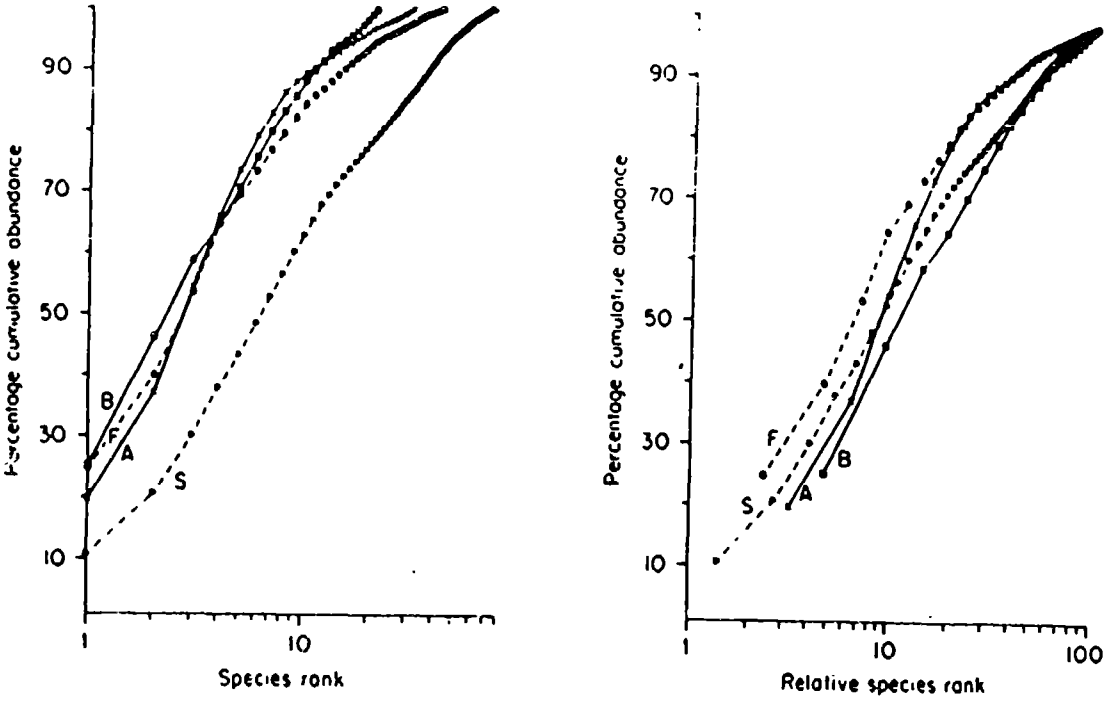


Fig. 8.5. k-dominance curves (left) and Lorenz curves (right) for 20 cm deep cores taken from experimental sand columns 20 days (A) and 77 days (B) after initial setup, and from intertidal (F) and subtidal (S) sand from the study site (from Lambshead et al., 1983).

ABUNDANCE / BIOMASS COMPARISON (ABC) PLOTS

The advantage of distribution plots such as k- dominance curves is that the distribution of species abundances among individuals and the distribution of species biomasses among individuals can be compared on the same terms. Since the two have different units of measurement, this is not possible with diversity indices.

This is the basis of the ABUNDANCE / BIOMASS COMPARISON (ABC) method of determining levels of disturbance (pollution-induced or otherwise) on benthic macrofauna communities. Both empirical evidence and theoretical considerations suggest that the k-dominance curve for biomass will fall above the curve for abundance in undisturbed (or unpolluted) communities, and vice versa for disturbed (or polluted) communities.

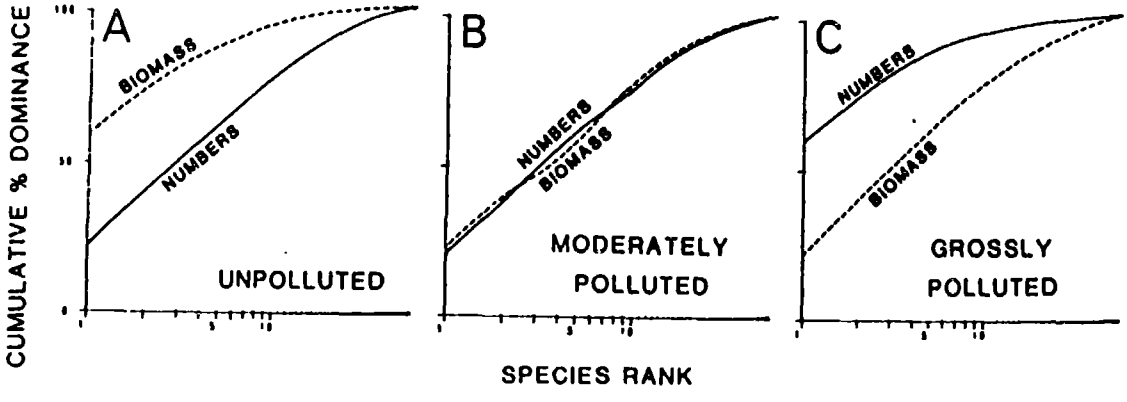


Fig. 8.6. Hypothetical k-dominance curves for species biomass and numbers, showing unpolluted, moderately polluted and grossly polluted conditions (from Warwick, 1986).

Example 1. Time series of macrobenthos in Loch Linnhe, Scotland in response to increasing and decreasing levels of organic enrichment (pulp-mill effluent). See Lecture 1, Figs. 1.3 and 1.4.

Example 2. Transect across sewage-sludge dumping ground at Garroch Head, Firth of Clyde, Scotland.

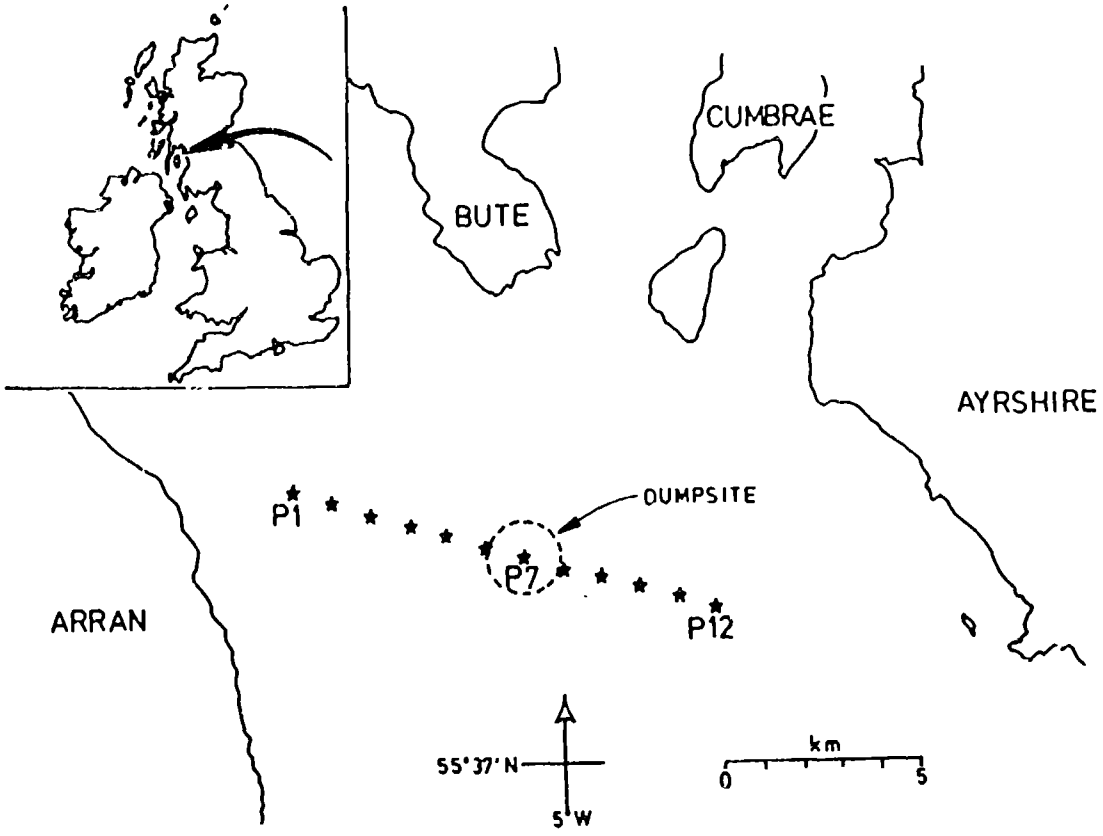


Fig. 8.7. Map showing location of dumping-ground. Centre of dump-site denoted by dashed circle: positions of sampling stations (P1 - P12) identified by asterisks.

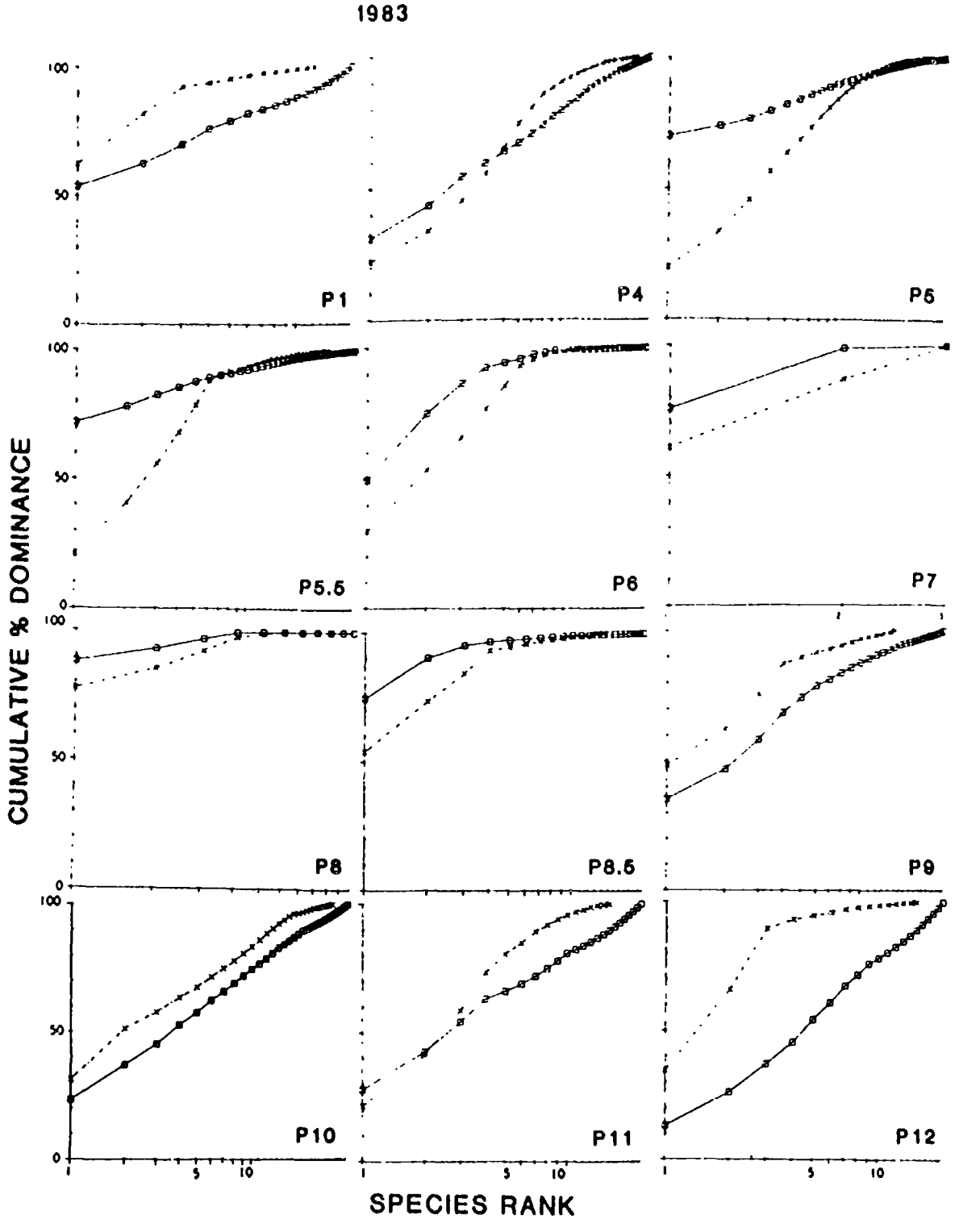


Fig. 8.8. ABC plots for macrobenthos on Garroch Head transect in 1983. Abundance = squares, biomass = crosses (From Warwick et al., 1987).

TRANSFORMATIONS OF k-DOMINANCE CURVES

PROBLEM: It is difficult to distinguish differences between k-dominance curves when cumulative frequencies are near 100% (sometimes after the first 2 or 3 spp.)

SOLUTION: Tranform y-axis so that cumulative values are close to linearity. Clarke (1990) suggests the modified logistic transformation:

$$y_i' = \log[(1 + y_i)/(101 - y_i)]$$

Example: Macrobenthos from Frierfjord / Langesundfjord, Norway (IOC/GEEP Oslo Workshop).

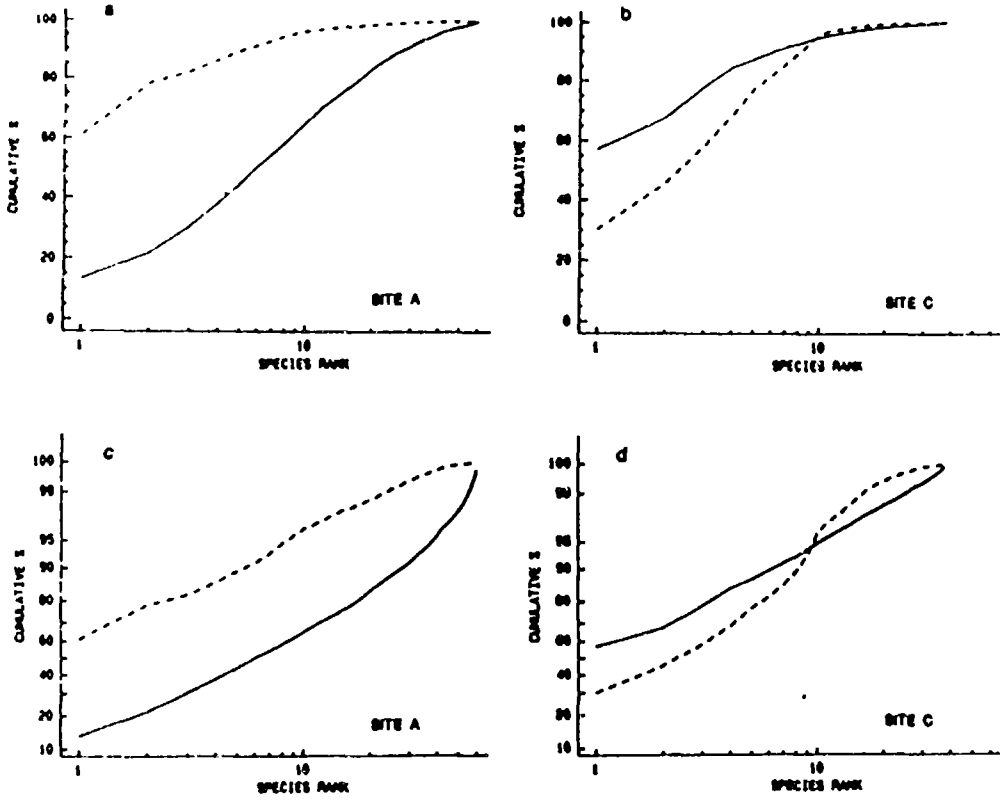


Fig. 8.9. a), b) Standard ABC plots for sites A (reference) and C (potentially impacted). c), d) ABC plots for sites A and C with the y-axis subjected to modified logistic transformation. Abundance = continuous line, biomass = dashed line.

PARTIAL DOMINANCE CURVES

PROBLEM: Visual information presented by k-dominance (and ABC) curves is over dependent on single most dominant species. Unpredictable presence of large numbers of small biomass species, or heavy spatfall of young of one species, may give false impression of disturbance.

SOLUTION: With genuine disturbance, patterns of ABC curves should be unaffected by successive removal of most dominant species in terms of abundance or biomass.
PARTIAL DOMINANCE CURVES (Clarke, 1990) compute the dominance of the second most dominant species over the remainder, the same with the third most dominant etc.

Example 1: Macrobenthos from Frierfjord/ Langesundfjord, Norway (IOC/GEEP Oslo Workshop).

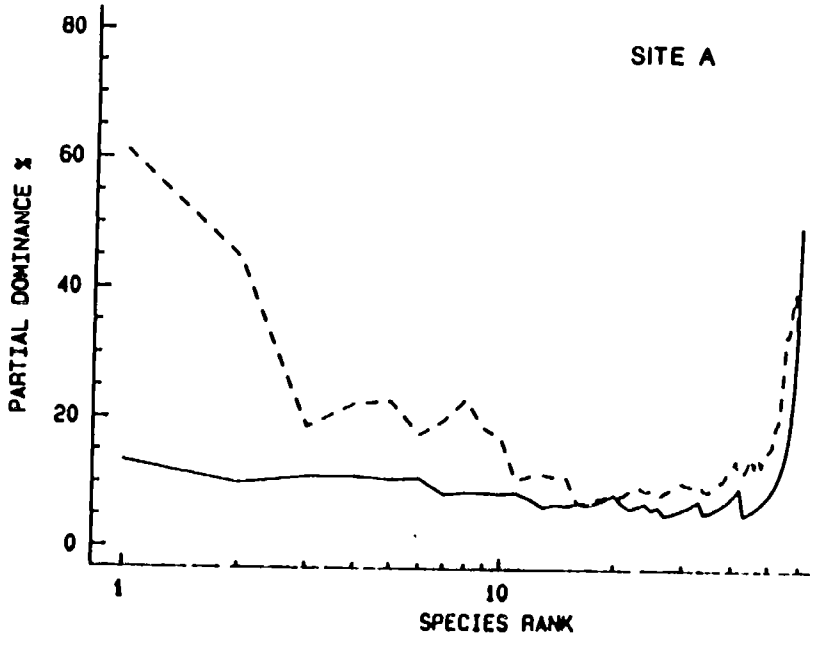


Fig. 8.10. Partial dominance curves (abundance/ biomass comparison) for reference station A (c.f. Figs 8.9a and c for corresponding standard and transformed ABC plots). This illustrates the typically undisturbed condition.

Example 2: Loch Linnhe macrobenthos, 1966-68, 1970-72.

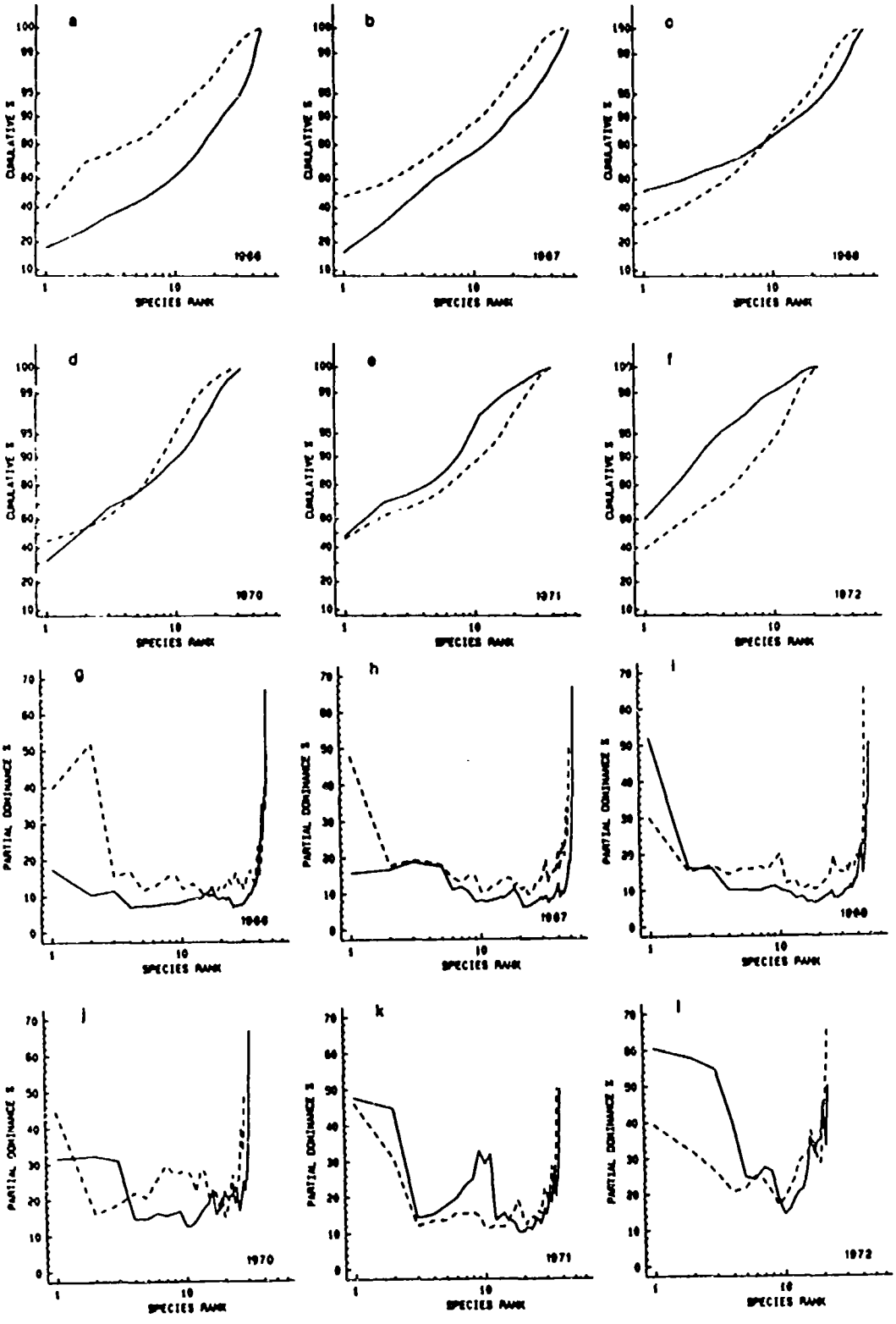


Fig. 8.11. a)-f) ABC curves (logistic transform). g)-l) Partial dominance curves for abundance (solid line) and biomass (dashed line) for the same years.

SIGNIFICANCE TESTING FOR GRAPHICAL METHODS

Given replicate curves (k-dominance, ABC, 'individuals amongst species' etc.) at 2 or more sites (or times etc.), need a TEST FOR SIGNIFICANT DIFFERENCE.

Example: Hamilton Harbour macrofauna.

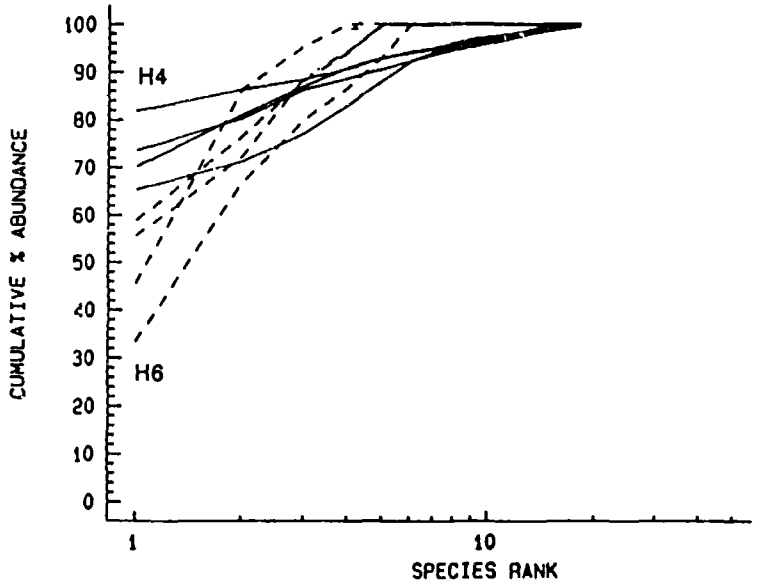


Fig. 8.12. Abundance k-dominance curves for four replicates at site H4 (solid) and H6 (dashed line).

Is the apparent difference for H4 and H6, in initial slope of curves, borne out statistically?

Also, testing for difference between sets of ABC CURVES at two (or more) sites reduces to a comparison of two (or more) sets of replicate curves by computing the DIFFERENCE CURVE B-A for each sample, e.g. Fig. 8.13.

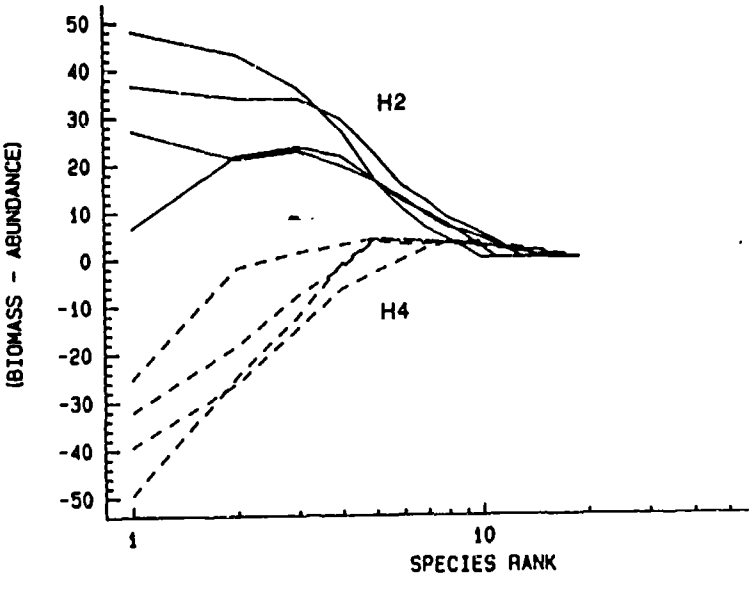


Fig. 8.13. Difference (B-A) between k-dominance curves for biomass and abundance for four replicate samples at H2 (solid) and H4 (dashed line).

FIRST APPROACH:

Reduce each replicate curve to a SINGLE SUMMARY STATISTIC. E.g. if $\{A_i\}$ and $\{B_i\}$ are the cumulative abundance and biomass values from an ABC plot (i=1,..,S species), define:

$$W = \Sigma_{i=1}^{S} (B_i - A_i)/[50(S-1)].$$

W takes values in (-1,1), with W→1 for totally even abundances across species but biomass dominated by a single species, and W→-1 for the converse case.

Similarly, for k-dominance curves of cumulative $\{A_i\}$:

$$K_A = [(\Sigma_{i=1}^{S} A_i) - 50(S+1)]/[50(S-1)],$$

where extremes are K→0 (evenness) and K→1 (dominance). K_B defined similarly for biomass.

Now, PERFORM ANOVA on SUMMARY STATISTICS (W or K) from each replicate (e.g. as for diversity indices). Works well in cases like Fig 8.13 (H2 & H4 differ significantly) but poorly for Fig. 8.12 where difference is in slope not mean area. Need more GENERAL TEST with power to detect any CONSISTENT DIFFERENCE between 2 (or more) sets of curves, so

SECOND APPROACH:

Define 'dissimilarity' between any pair of curves $\{A_{i1}; i=1,..,S_1\}$, $\{A_{i2}; i=1,..,S_2\}$, as their total (absolute) distance apart:

$$d = \Sigma_{i=1}^{S_{max}} |A_{i1} - A_{i2}|$$

where $S_{max} = \max(S_1, S_2)$. Or better reflection of visual difference in two k-dominance curves is:

$$d' = \Sigma_{i=1}^{S_{max}} |A_{i1} - A_{i2}| \log(1+i^{-1}).$$

Compute d (or d') for every pair of replicate curves, to give lower triangular dissimilarity matrix, and CALCULATE ANOSIM STATISTIC R, eqt. (6.1).

PERMUTATION/RANDOMIZATION TEST of difference between sites/times etc. then carried out exactly as in lecture 6. (ANOSIM on Fig. 8.12 distinguishes H4 and H6, whereas ANOVA on K_A does not).

Details in Clarke (1990). Note that principle EXTENDS TO OTHER GRAPHICAL METHODS, e.g. partial dominance, 'individuals amongst species' curves etc.

LECTURE 9

TRANSFORMATIONS

There are two distinct roles for transformations in community analysis:

a) to validate assumptions for parametric analyses - applies to UNIVARIATE tests

b) to weight the contributions of common and rare species in a MULTIVARIATE representation.

UNIVARIATE

Example: Frierfjord macrofauna. Indicator species.

Site:	A	B	C	D	E	G
Replicate						
1	1	7	0	1	62	66
2	4	0	0	8	102	68
3	3	3	0	5	93	52
4	11	2	3	13	69	36
Mean	4.8	3.0	0.8	6.8	81.8	55.5
Stand. dev.	4.3	2.9	1.5	5.1	13.7	14.8

Table 9.1. Thyasira sp. numbers in 4 replicate grabs at 6 sites.

NOTE:

1) The replicates are not symmetrically distributed (they tend to be right-skewed), so normality assumptions are dubious.

2) More importantly (for test validity), the variance increases strongly with the mean - this invalidates "constant variance" assumptions of ANOVA.

Both problems can be tackled by:

POWER TRANSFORMATION:

Individual replicates y are transformed to y*, given by:

$$y^* = (y^\lambda - 1)/\lambda \qquad (9.1)$$

where, in order of INCREASING SEVERITY,

$\lambda = 1$ - no transform
$\lambda = 0.5$ - square root ($\sqrt{}$)
$\lambda = 0.25$ - 4th root ($\sqrt{\sqrt{}}$)
$\lambda \to 0$ - log transform ($y^* = \log_e y$)

Possible to determine best λ, anywhere in (0,1), for each separate data set (Box and Cox 1964), but unnecessarily precise - better just to choose between above 4 cases, using:

TAYLOR'S POWER LAW:

If:

$$\text{var}(y) \propto (\text{mean } y)^{\nu} \qquad (9.2)$$

then:

$$\text{var}(y^{\lambda}) \propto (\text{mean})^{2(\lambda-1)+\nu} \text{ (approx.)} \qquad (9.3)$$

Choose $\lambda = 1-(\nu/2)$ to get var(y) = constant.

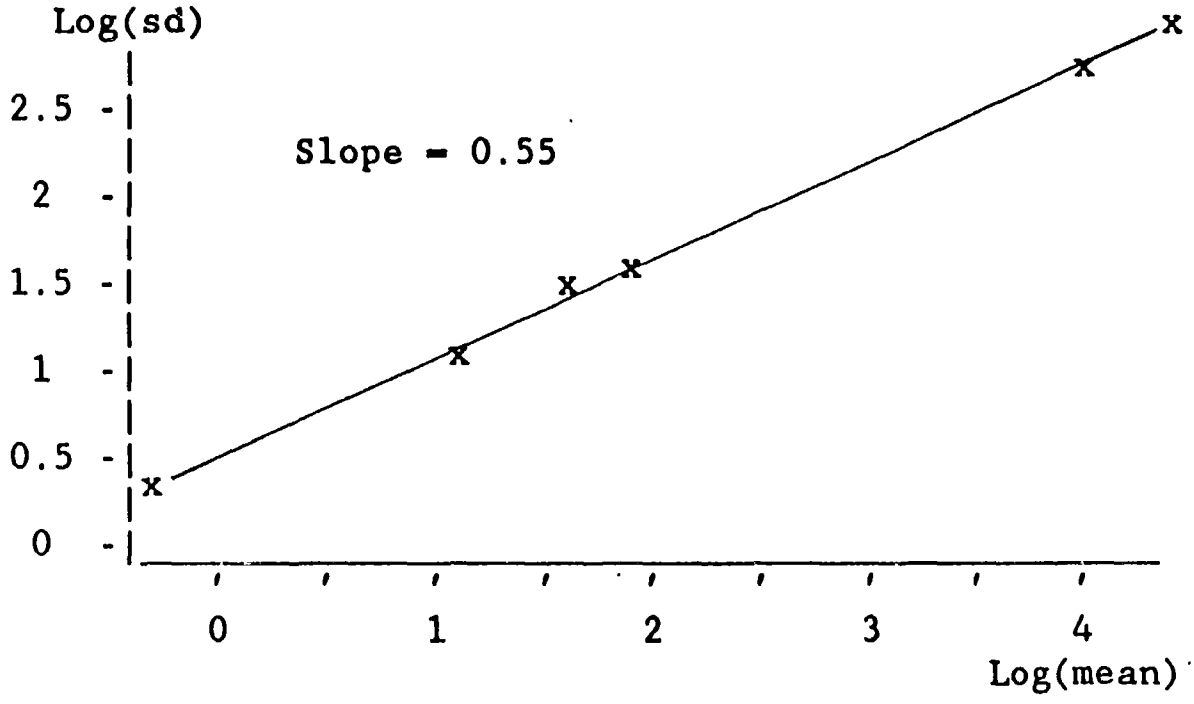
Find ν by regressing log (stand. dev.) on log (mean), because:

$$\log(\text{sd}(y)) = (\nu/2)\log(\text{mean } y) + \text{constant} \qquad (9.4)$$

So $\lambda = 1$ - (slope of regression), thus if:

slope = 0 - no transform
= 0.5 - use $\surd$
= 0.75 - use $\surd\surd$
= 1 - use $\log_e$ (9.5)

Example: Thyasira numbers at 6 sites



Plot indicates $\surd$ appropriate. After transform:

Site	A	B	C	D	E	G
Mean(y*)	2.01	1.45	0.43	2.42	9.00	7.40
Sd(y*)	0.97	1.10	0.87	1.10	1.04	1.04

VARIANCE STABILIZED so ANOVA and follow-up tests VALID (show E,G different from the rest, clearly). Means and confidence intervals should be back-transformed to original scales (intervals not symmetric but then data was not symmetric).

CAUTION: Beware of doing multiple ANOVAs on a range of indicator species (each runs a 5% risk of error and this compounds). Alright if performed (at higher significance) on a few species selected a priori.

AVOID "SNOOPING" in a large data array for likely species to do an ANOVA on; certain to find some which are significant, even in a random array !

MULTIVARIATE

TRANSFORMS can be used for the same reason as in univariate analyses - to induce (multivariate) normality, e.g. for MANOVA tests (lecture 6), but: a) Insufficient to demonstrate univariate normality and constant variance (for each variable) to prove multivariate normality and constant covariance.
b) Rarely possible to achieve (marginal) normality for species abundance/biomass data (though possible for, say, a matching set of diversity indices).

MORE IMPORTANT USE OF TRANSFORMS IN COMMUNITY DATA is in WEIGHING rare and common species in forming similarities between sites, e.g. Bray-Curtis:

$$S_{jk} = 100\left(1 - \frac{\Sigma_{i=1}^{p} |y_{ij} - y_{ik}|}{\Sigma_{i=1}^{p} (y_{ij} + y_{ik})}\right) \qquad (9.6)$$

Example: Loch Linnhe macrofauna, subset

Sample:	1	2	3	4
Species				
Echinoca.	9	0	0	0
Myrioche.	19	0	0	3
Labidopl.	9	37	0	10
Amaeana	0	12	144	9
Capitella	0	128	344	2
Mytilus	0	0	0	0

UNTRANSFORMED

Sample	1	2	3	4
1	-			
2	8	-		
3	0	42	-	
4	39	21	4	-

Sample:	1	2	3	4
Species				
Echino.	1.7	0	0	0
Myrioc.	2.1	0	0	1.3
Labido.	1.7	2.5	0	1.8
Amaena	0	1.9	3.5	1.7
Capite.	0	3.4	4.3	1.2
Mytilus	0	0	0	0

√√ TRANSFORMED

Sample	1	2	3	4
1	-			
2	26	-		
3	0	68	-	
4	52	68	42	-

Untransformed similarities are lower (unimportant in itself since MDS is only a function of ranks) but RANK SIMILARITIES ARE TOTALLY CHANGED by transform.

Untransformed similarities are DOMINATED BY THE COMMONEST SPECIES, e.g. comparing samples 2 and 4 and omitting each species in turn:

Species omitted:	None	1	2	3	4	5	6
Bray-Curtis (S):	21	21	21	14	13	54	21

By contrast, under a √√ transform, ALL (present) SPECIES MAKE SOME CONTRIBUTION to the similarity:

Species omitted:	None	1	2	3	4	5	6
Bray-Curtis (S):	68	68	75	61	59	76	68

TRANSFORMATION SEQUENCE:

None –> $\sqrt{}$ –> $\sqrt{\sqrt{}}$ –> log –> Presence/absence

puts PROGRESSIVELY LESS WEIGHT on common species and increasingly takes account of rarer ones.

Logical end-point is REDUCTION of the data array to one of PRESENCE OR ABSENCE OF SPECIES (this is a transformation to the numbers 0 or 1), where all species contribute equally.

Example: Loch Linnhe macrofauna, subset.

Sample:	1	2	3	4
Species				
Echinoca.	1	0	0	0
Myrioche.	1	0	0	1
Labidopl.	1	1	0	1
Amaena	0	1	1	1
Capitella	0	1	1	1
Mytilus	0	0	0	0

PRESENCE/ABSENCE

Sample	1	2	3	4
1	-			
2	33	-		
3	0	80	-	
4	57	86	67	-

NOTE: 1) NEED TO USE log(1+y) not log y DISTORTS TRANSFORM SEQUENCE. log(1+y) intermediate between $\sqrt{\sqrt{}}$ and presence/absence for moderate or large counts but less severe than $\sqrt{\sqrt{}}$ for small counts.

2) $\sqrt{\sqrt{}}$y preferred to log(1+y) because Bray-Curtis is INVARIANT TO A SCALE CHANGE (eg. for biomass) if $\sqrt{\sqrt{}}$ is used. (Little difference in practice though).

3) As severity of transform increases, more species contribute, so sample relationships are expressed in higher-dimensional space, and ordination in 2-d is harder (eg. Fig. 9.1). So, WRONG to assume that TRANSFORMS GIVING LOWER STRESS ARE BETTER; the converse is true if added species are important.

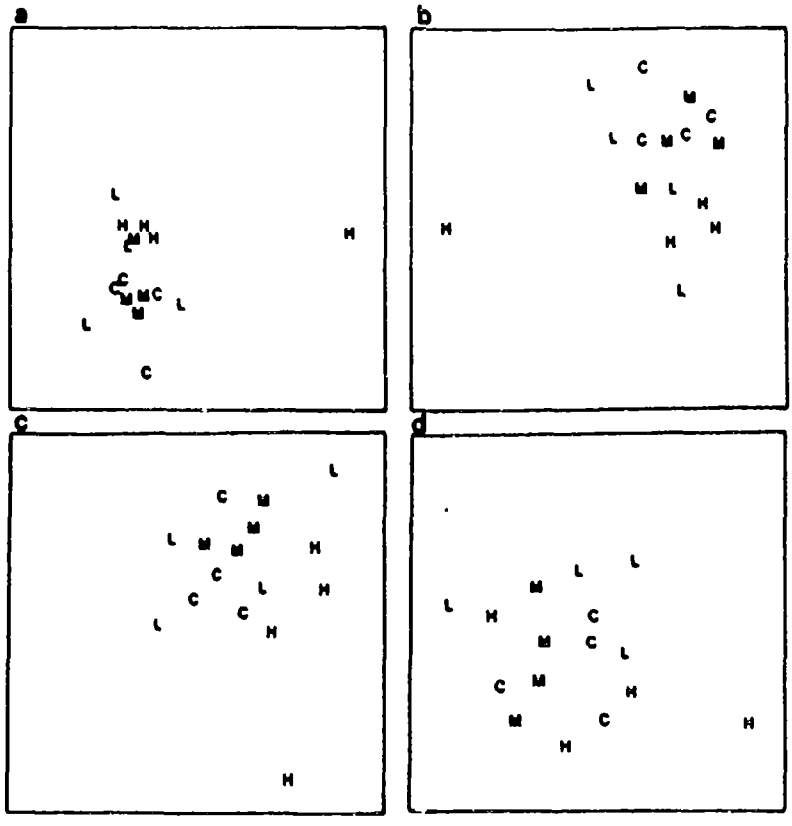


Fig. 9.1. GEEP mesocosm nematodes (Warwick et al. 1988). MDS of 4 boxes from 4 treatments (C,L,M,H). Bray-Curtis similarities from transformed counts: a) no transform, b) $\sqrt{}$, c) $\sqrt{\sqrt{}}$, d) presence/absence. Stress: a) 0.08, b) 0.14, c) 0.19, d) 0.19.

4) SAME TRANSFORM SEQUENCE APPLIES TO PCA (and other ordinations) with much the same consequences.

5) Log (or $\sqrt{\sqrt{}}$) transforms effectively REDUCE DATA TO A 6 POINT SCALE, i.e. 0 = absent, 1 = one individual, 2 = handful, 3 = sizeable, 4 = abundant, 5 = very abundant; replacing data by this scale will make no real difference to the multivariate displays. This may appear crude but often genuinely reflects inherent variability, so greater accuracy in counting may be unnecessary.

CONCLUDE:

1) CHOICE OF TRANSFORM often has a bigger effect on conclusions than the CHOICE OF ORDINATION method.

2) "What is the RIGHT TRANSFORM for a multivariate analysis?" is largely a BIOLOGICAL rather than a STATISTICAL question (unlike the use of transforms for validating assumptions); the choice of transform determines how the similarity of two samples is defined.

RECOMMEND:

Use INTERMEDIATE transform (eg. $\sqrt{}$, $\sqrt{\sqrt{}}$ or LOG) rather than either of the two EXTREMES:

a) NO TRANSFORM - MDS reflects only 2 or 3 commonest species, so INTERPRETATION is likely to be SHALLOW.

b) PRESENCE/ABSENCE - CHANCE OCCURRENCES of rare species DOMINATE the SAMPLE RELATIONSHIPS in high dimensions and make it difficult to get an interpretable low-dimensional ordination.

LECTURE 10

SPECIES REMOVAL AND AGGREGATION

SPECIES REMOVAL

Two reasons for ELIMINATING SPECIES discussed earlier:

a) For sample PCA (not MDS) ordination, must reduce to (say) <50 species, else problems with eigenvalues.

b) For species ordinations, though MDS and CLUSTER are possible for all species, rarer (chance) species must be excluded for an interpretable outcome.

RECOMMEND retaining species accounting for >p% OF TOTAL SCORE (abundance or biomass) in ANY ONE SAMPLE (p chosen to reduce to required number, typically p = 3 or 4). Allows for high diversity/ low abundance samples which could have all species eliminated by simple selection of the top q% most abundant species over all samples.

SPECIES REDUNDANCY: Since sample relationships can often be well summarised in a 2-d ordination (from, say, a 100-d species space), many SPECIES MUST BE INTERCHANGEABLE in the way they characterise the samples. This can be seen by performing MDS on a randomly chosen subset (say 20%) of species:

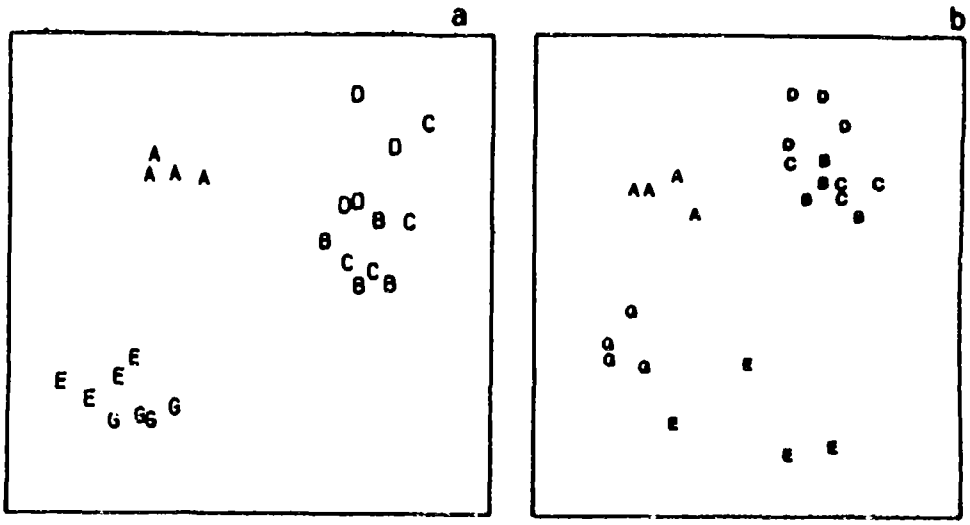


Fig. 10.1. Frierfjord macrofauna counts. Sample MDS (Bray-Curtis, √√) for: a) all 110 species, b) 19 random species. (Stress: a) 0.14, b) 0.13).

Above example of no practical interest, but suggests:

SPECIES AGGREGATION to higher taxonomic levels.

If results from identifications to higher taxonomic levels are comparable to a full species analysis:

a) a great deal of LABOUR CAN BE SAVED;

b) LESS FAUNAL EXPERTISE NEEDED - major factor in parts of the world where fauna is poorly described.

METHODS AMENABLE TO AGGREGATION:

1) MULTIVARIATE:

All ordination/clustering techniques.

Empirical evidence is increasing that identification only to family level makes little difference.

2) DISTRIBUTIONAL:

a) Aggregation for ABC curves is possible; family level analyses are often identical to species level analyses (see Figs. 10.6 and 10.7).

b) Untried for other methods (eg. individuals amongst species curves).

3) UNIVARIATE:

a) Concept of "indicator groups" is well-established (eg. nematode/copepod ratios).

b) Can define diversity indices at hierarchical taxonomic levels (though not commonly used in practice).

Warwick (1988) hypothesises further motivation: that pollution may change community composition at higher taxonomic levels (eg. phyla) whereas natural variables (grain size, water depth etc.) modify it more by species replacement (within phyla). Thus, distribution of higher taxa may even relate more closely to the contamination gradient than species data, the latter being more complicated by effects of confounding natural variables.

MULTIVARIATE EXAMPLES

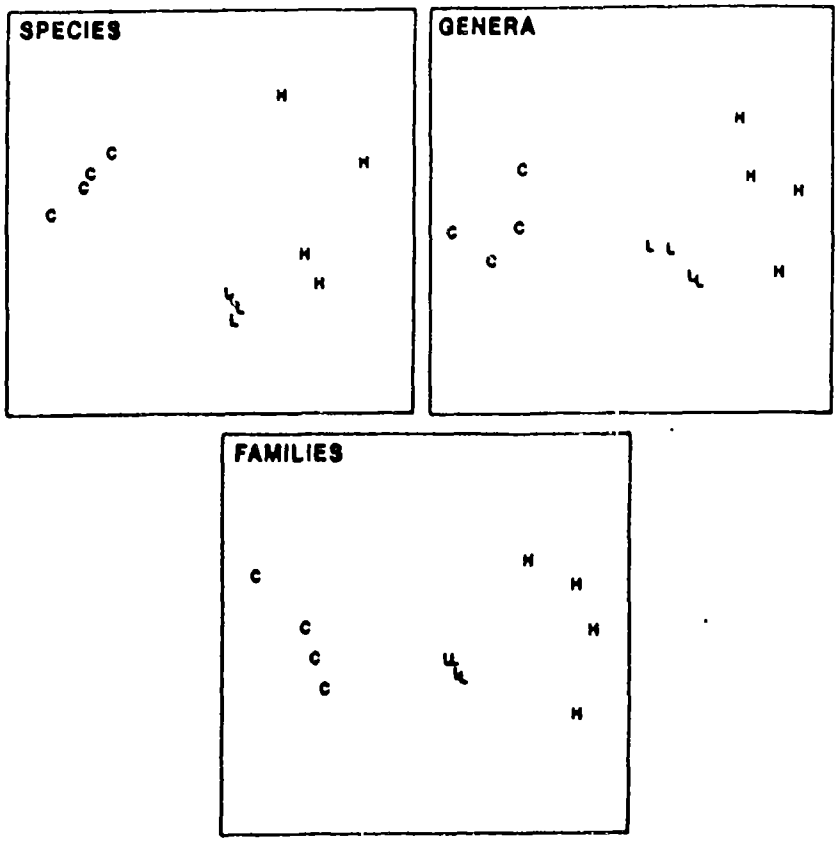


Fig. 10.2. Mesocosm copepod counts - 3 levels of nutrient enrichment (Gee et al. 1985). Sample MDS plot (Bray-Curtis, $\sqrt{\sqrt{}}$ transform); species data aggregated into genera and families (Warwick 1988).

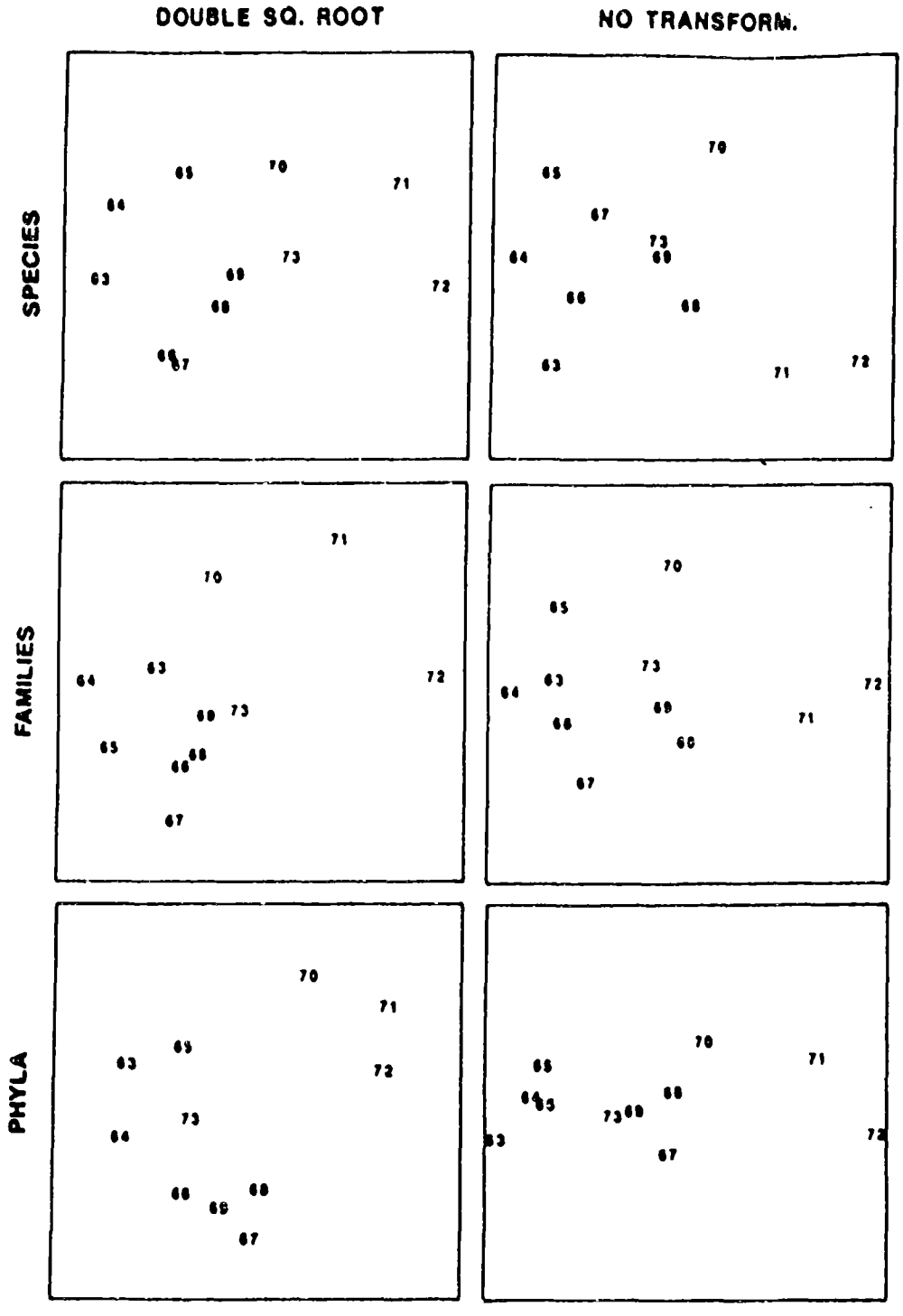


Fig. 10.3. Loch Linnhe macrofauna (Pearson 1975). MDS (Bray-Curtis) of 11 years samples for $\sqrt{\sqrt{}}$ transform (left) and no transform (right), based on abundances from 115 species (top), aggregated into 45 families (middle) and 9 phyla (bottom), Warwick (1988). Note more linear configuration for phyla.

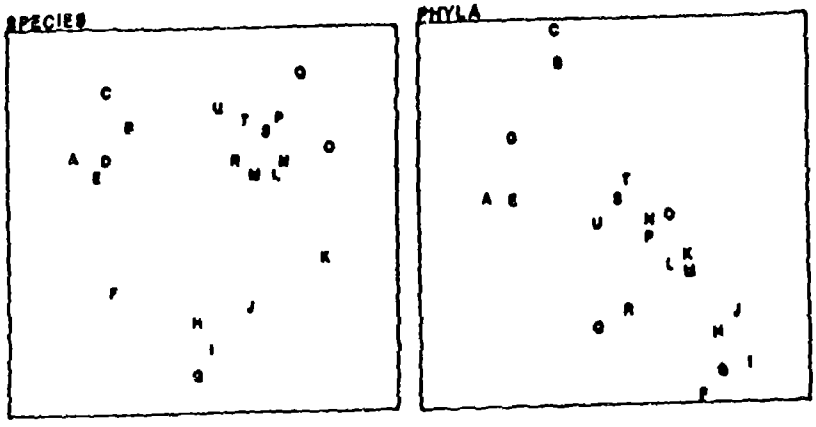


Fig. 10.4. MDS for macrobenthos at station "Pierre Noire". Species data (left) aggregated into phyla (right). Sampling months are A:4/77, B:8/77, C:9/77, D:12/77, E:2/78, F:4/78, G:8/78, H:11/78, I:2/79, J:5/79, K:7/79, L:10/79, M:2/80, N:4/80, O:8/80, P:10/80, Q:1/81, R:4/81, S:8/81, T:11/81, U:2/82.

Oil-spill was during 3/78, i.e. between E and F. Note more linear configuration for phyla.

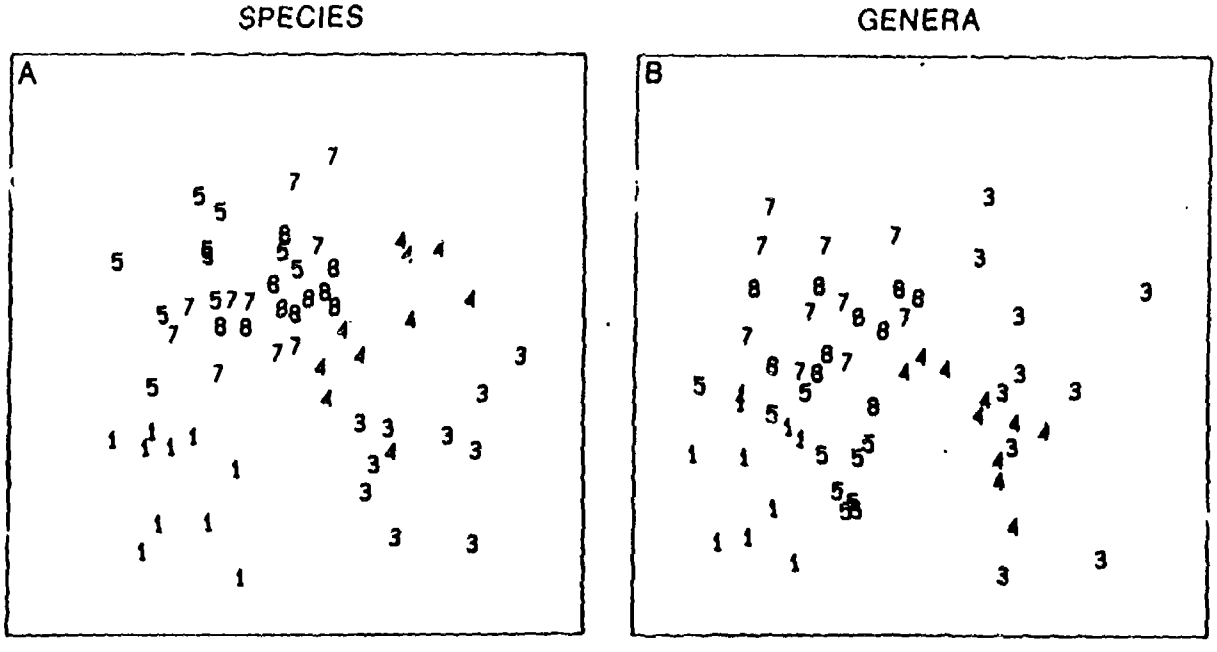


Fig. 10.5. MDS for coral species (n=75) and genus (n=24) cover data at South Pari Island, Indonesia. El Niño occurred in 1982-3. 1=1981, 3=1983 etc.

GRAPHICAL/DISTRIBUTIONAL EXAMPLES

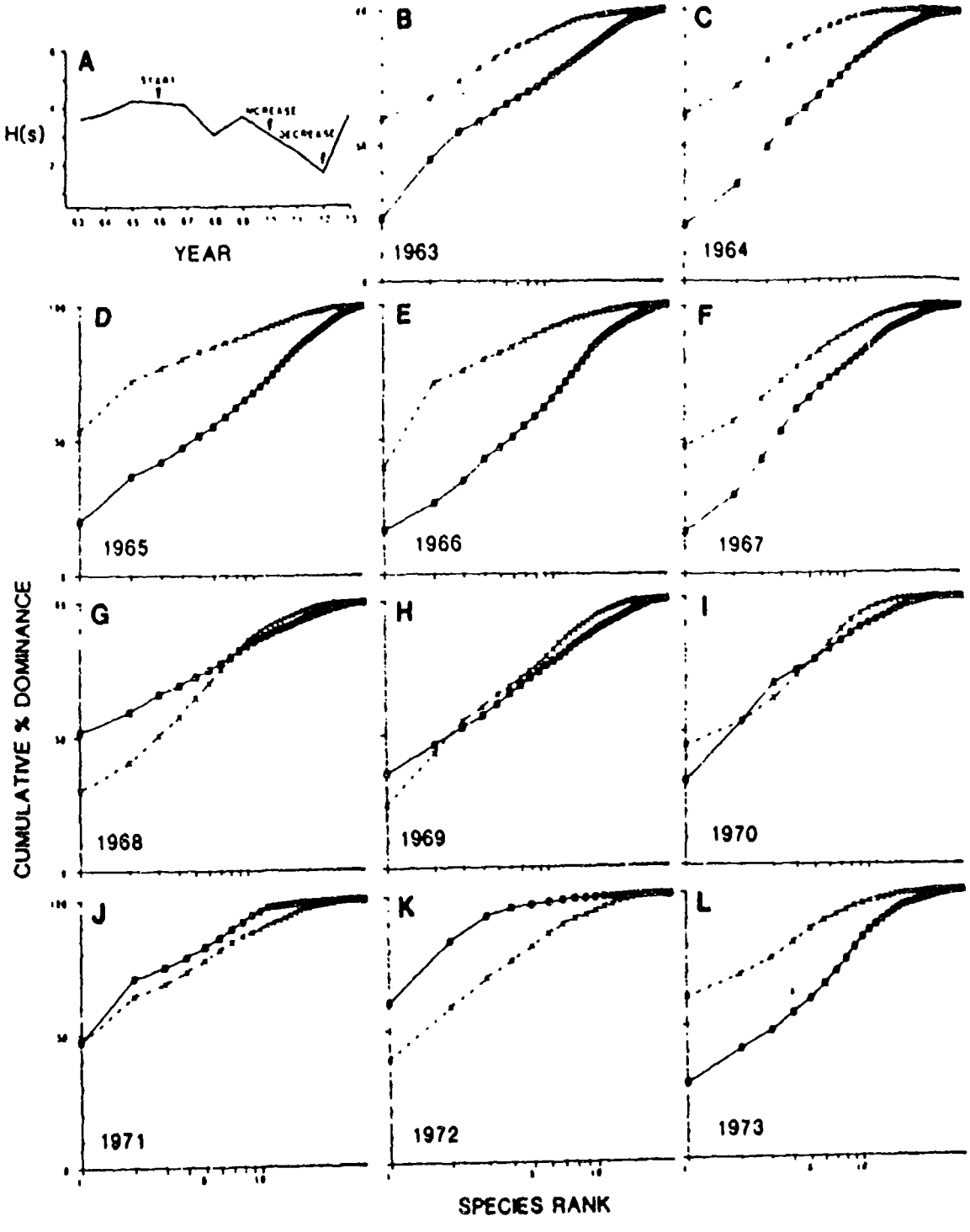


Fig. 10.6. Loch Linnhe macrofauna. (A) Diversity H', (B)-(L) "ABC" curves for 11 years, of biomass (crosses) and abundance (squares). Analysis at species level, Warwick (1986).

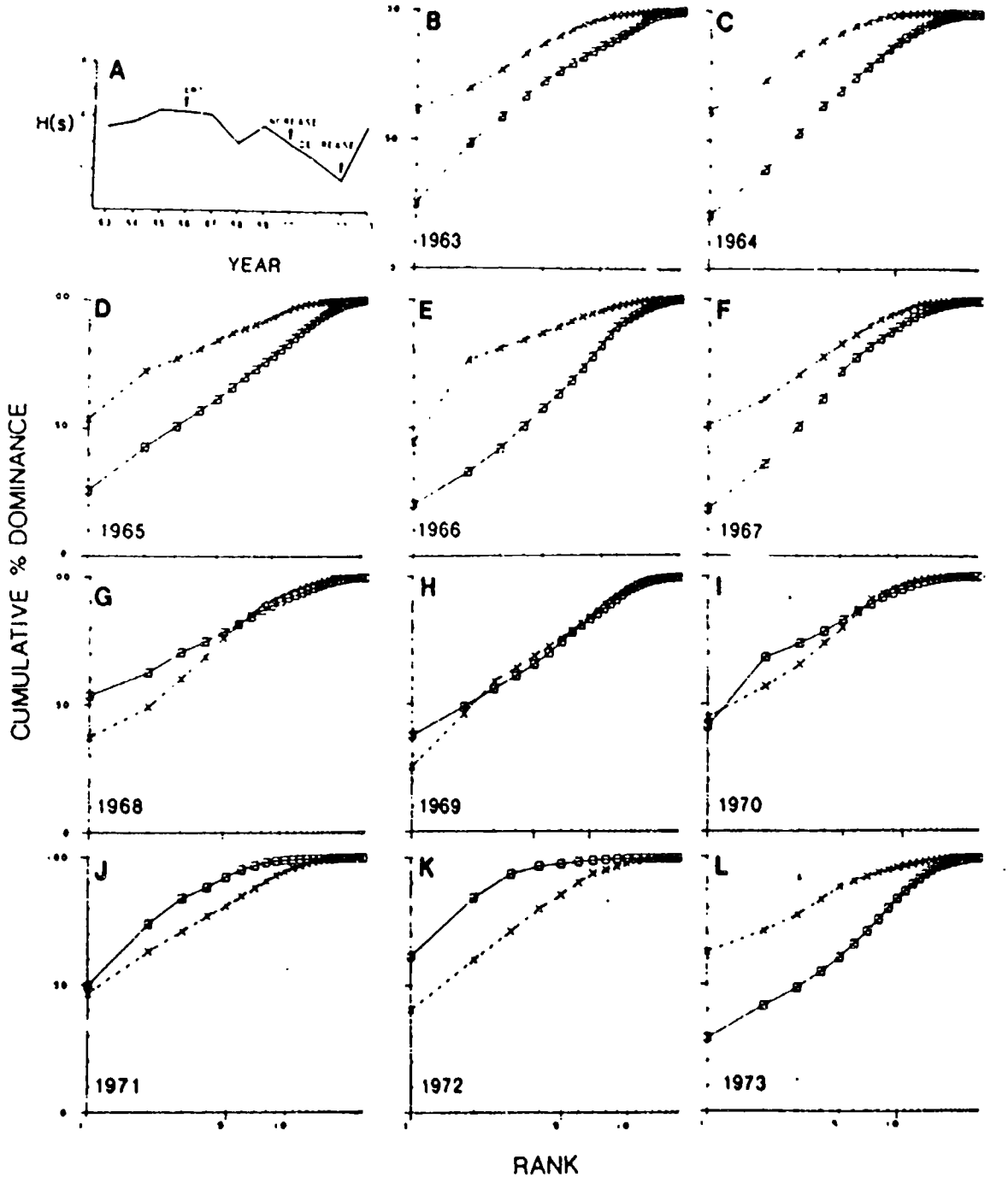


Fig. 10.7. Loch Linnhe macrofauna. (A) Diversity H', (B)-(L) "ABC" curves for 11 years, of biomass (crosses) and abundance (squares), for data aggregated to families, Warwick (1988).

UNIVARIATE EXAMPLE

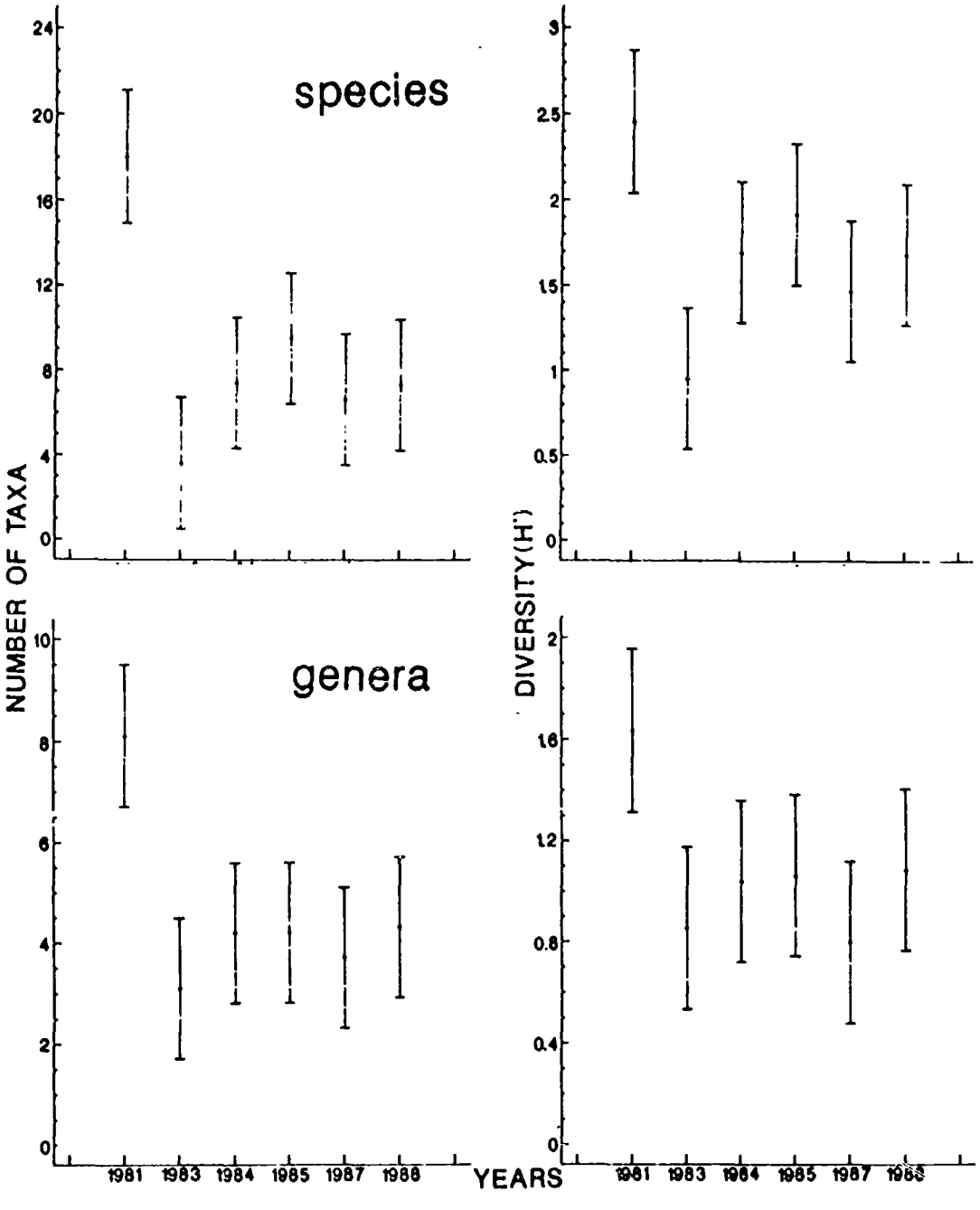


Fig. 10.8. Plots of number of taxa and Shannon diversity for reef corals at South Tikus Island, Indonesia, showing impact and partial recovery from 1982-3 El Ni-ɳo. Species data (upper) have been aggregated into genera (lower). Note similarity of patterns.

LECTURE 11

LINKING MULTIVARIATE AND UNIVARIATE COMMUNITY ANALYSES TO ENVIRONMENTAL VARIABLES

APPROACH

1) FAUNAL AND ENVIRONMENTAL ANALYSIS SEPARATED intially, i.e. the biota is allowed to "tell its own story" without the use of physical or chemical data:

   a) to DEMONSTRATE the RELATIONSHIPS between samples and differences (if any) between sites (/times),

   b) to INFER COMMUNITY DISTURBANCE at some sites.

2) ENVIRONMENTAL VARIABLES ANALYSED ON THEIR OWN, for similar reasons. Two classes of variables:

"NATURAL" PHYSICAL (or "background") VARIABLES, such as depth of the water column, sediment granulometry, salinity, etc. and

CONTAMINANT VARIABLES, measuring chemical impact.

Analysis attempts:

   a) to DEMONSTRATE DIFFERENCES (if any) in physical or chemical variables between the sites,

   b) to REDUCE the COMPLEXITY of the environmental measures, particularly the chemical data, so the nature of the impact (if any) can be summarised by a few key variables.

3) SUMMARY REPRESENTATIONS of both biological and environmental analyses are VIEWED TOGETHER:

   a) to examine whether changes between sites (/times) seem to be the product of differences in "natural" environmental variables, or

   b) are correlated with inferred or measured contaminant impact.

ANALYSIS OF ENVIRONMENTAL DATA

UNIVARIATE: Background (physical) variables are typically univariate, with little variability between replicates within a site (e.g. water depth).

Where there is variability, and it is helpful to establish site differences, use ANOVA and confidence intervals (e.g. as for diversity).

MULTIVARIATE: Chemical measurements can often be highly multivariate (e.g. wide range of PAH compounds, PCB congeners, heavy metals etc.)

Example: Frierfjord sediment - heavy metals.

Site	Cu	Zn	Pb	Ni	Cr	Cd	Mn	Fe
A	28	141	73	33	40	0 8	454	3 5
	26	139	71	30	40	(0 6)	653	3 3
	27	147	67	29	35	(0 6)	503	3 1
B	48	238	134	33	50	(0 6)	1 050	3 5
	47	228	130	32	50	1 1	2 880	3 5
	64	297	167	32	40	1 1	664	3 1
C	44	228	135	35	51	0 8	1 500	4.1
	42	216	126	35	60	0 8	3 570	4 2
	42	208	117	33	45	1.1	5 880	4 0
D	48	241	142	37	56	0.9	1 720	4 3
	39	205	114	33	50	0.8	8 480	4 4
	44	238	141	35	34	1 1	5 440	4 1
E	38	199	160	22	40	0 8	484	2 2
	40	241	156	25	40	1 1	925	2.1
	107	275	184	28	45	1 1	1 400	2 5
F	48	328	118	32	35	3 6	10 380	3 1
	44	296	110	30	35	3 1	5 880	3 0
	47	320	118	32	35	3 4	7 430	3 0
G	67	349	212	35	61	2 2	1 060	2 8
	70	357	229	35	66	2 5	638	2 7
	77	417	267	38	70	4 5	619	2 6

Table 11.1. Frierfjord sediments. Metal concentrations ($\mu g/g$ dry wt, Fe as %) in top 2 cm from 3 replicate cores at sites A-E,G. Abdullah & Steffenak (1988).

SAME RANGE OF MULTIVARIATE METHODS AVAILABLE as for faunal analyses (replace species by chemical "species"). However, type of data is different:

a) ZEROS do NOT predominate,

b) distribution NOT highly RIGHT-SKEWED.

c) REDUNDANCY can be very extreme, i.e. similar chemical compounds correlate very closely with each other along a spatial contaminant gradient.

So, possibly after (mild) TRANSFORMATION (e.g. $\sqrt{}$),

a) MULTIVARIATE NORMAL assumptions often justified;

b) PCA is useful, a 2-d ordination often giving a good representation of site chemistry,

c) *TESTING* of site differences can either be by MANOVA (e.g. Wilks'Λ) or by ANOSIM on a Euclidean distance dissimilarity matrix.

Example: Frierfjord sediment metals.

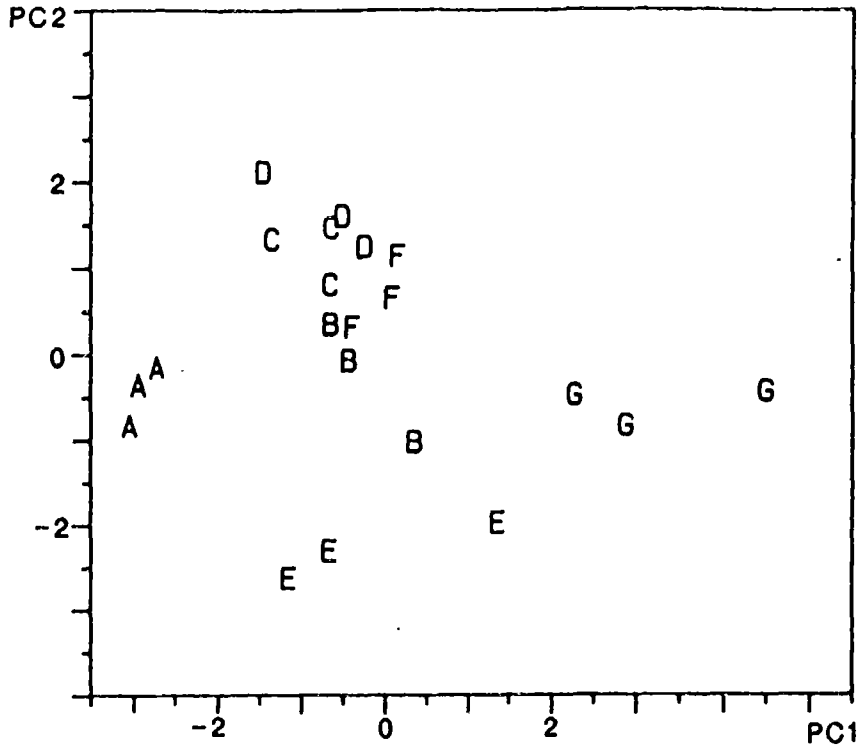


Fig. 11.1. Frierfjord sediments. 2-d PCA of metal data of Table 11.1 (√-transformed and normalised).

NOTE, in Fig. 11.1:

1) First 2 PCs ACCOUNT FOR 69% OF VARIABILITY, so 2-d ordination is not too bad a representation.

2) Some DIFFERENCES BETWEEN SITES ($p<0.001$ in ANOSIM test), principally between A, G and the rest.

3) PC1 represents an AXIS OF INCREASING CONTAMINANT LOAD, the weights given to the (normalised) Cu, Zn, Pb, Ni, Cr, Cd, Mn, Fe levels being 0.41, 0.48, 0.46, 0.30, 0.35, 0.35, -0.05 and -0.21.

4) PC1 AXIS is thus a UNIVARIATE descriptor of the overall metal load, useful in relating this chemistry to faunal descriptions.

5) Though it exists, the CONTAMINANT GRADIENT is WEAK, no more than a factor of 2 or 3 between the extremes, A and G. (PAH gradient weaker still).

RELATION TO FAUNAL ANALYSES - FIRST APPROACH

SELECT at most 2 or 3 DESCRIPTORS of the CONTAMINANT GRADIENT (eg. one for metals, one for hydrocarbons) - even 2 or 3 could be ambitious if the different classes of contaminants are well-correlated.

The two cases considered below are when the biological data are UNIVARIATE (eg. diversity indices) and when they are MULTIVARIATE (eg. ordinations).

UNIVARIATE

REGRESSION is a possible technique: either

SIMPLE LINEAR REGRESSION (1 environmental variable) or MULTIPLE LINEAR REGRESSION (for 2 or more)

or NON-LINEAR REGRESSION (if there is a range of contaminant values and sufficient replicates to justify a more complex "dose-response" curve.)

Example: Frierfjord macrofauna.

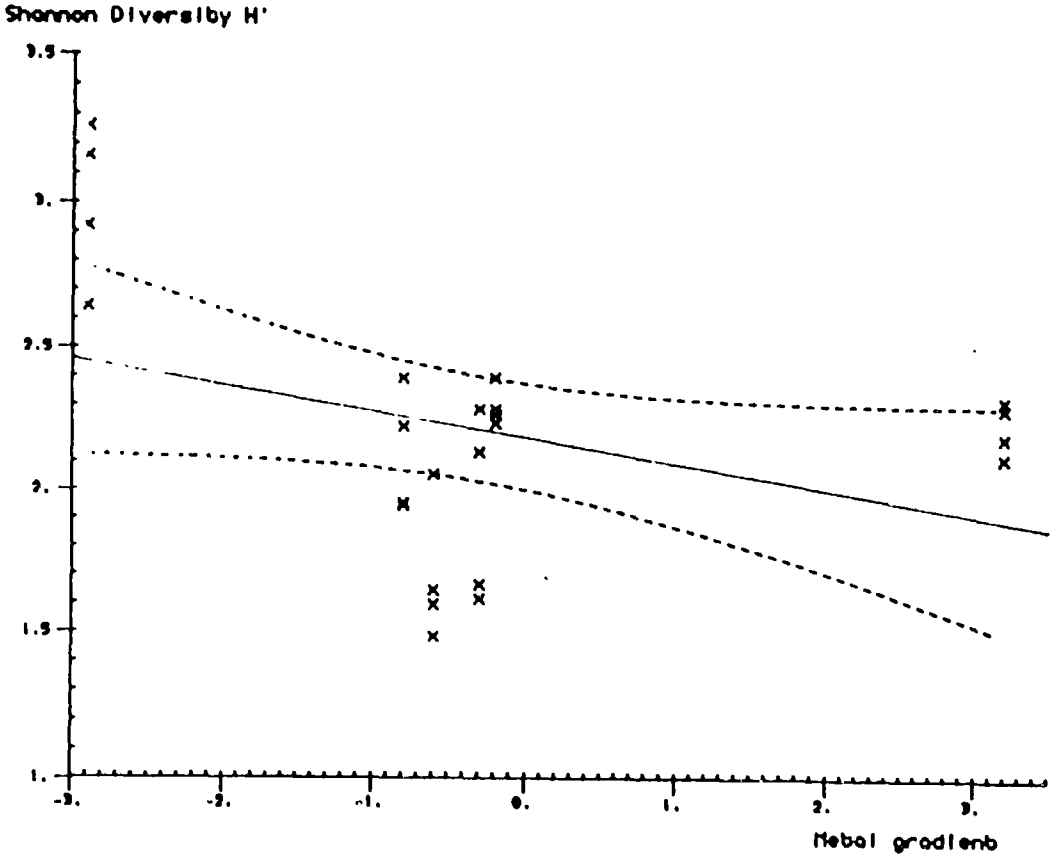


Fig. 11.2. Frierfjord macrofauna abundances. Shannon diversity H' regressed on an overall measure of sediment metal concentration (latter is mean PC1 at each of the 6 sites, from the PCA of Fig. 11.1).
x - replicate grabs, – fitted regression line,
--- 95% confiden/ce "funnel" for the mean H' at any metal concentration.

NOTE: Simple linear regression of H' on metal levels is not convincing!

a) Slope just fails to differ signficantly from zero, at 5%).

b) Linear relation is not adequate (but data does not justify more complex fit).

c) Most prominent feature (clear from the earlier ANOVA also - Fig. 6.1) is the general drop in diversity from the "reference" site (A).

MULTIVARIATE

SUPERIMPOSITION OF ENVIRONMENTAL VARIABLES ON FAUNAL ORDINATION: an effective visual technique performed separately for each environmental variable.

This may allow a GRADIENT in the ENVIRONMENTAL VARIABLE to be matched visually to a GRADIENT of change in the COMMUNITY structure.

Example: Bristol Channel zooplankton, April 1978.

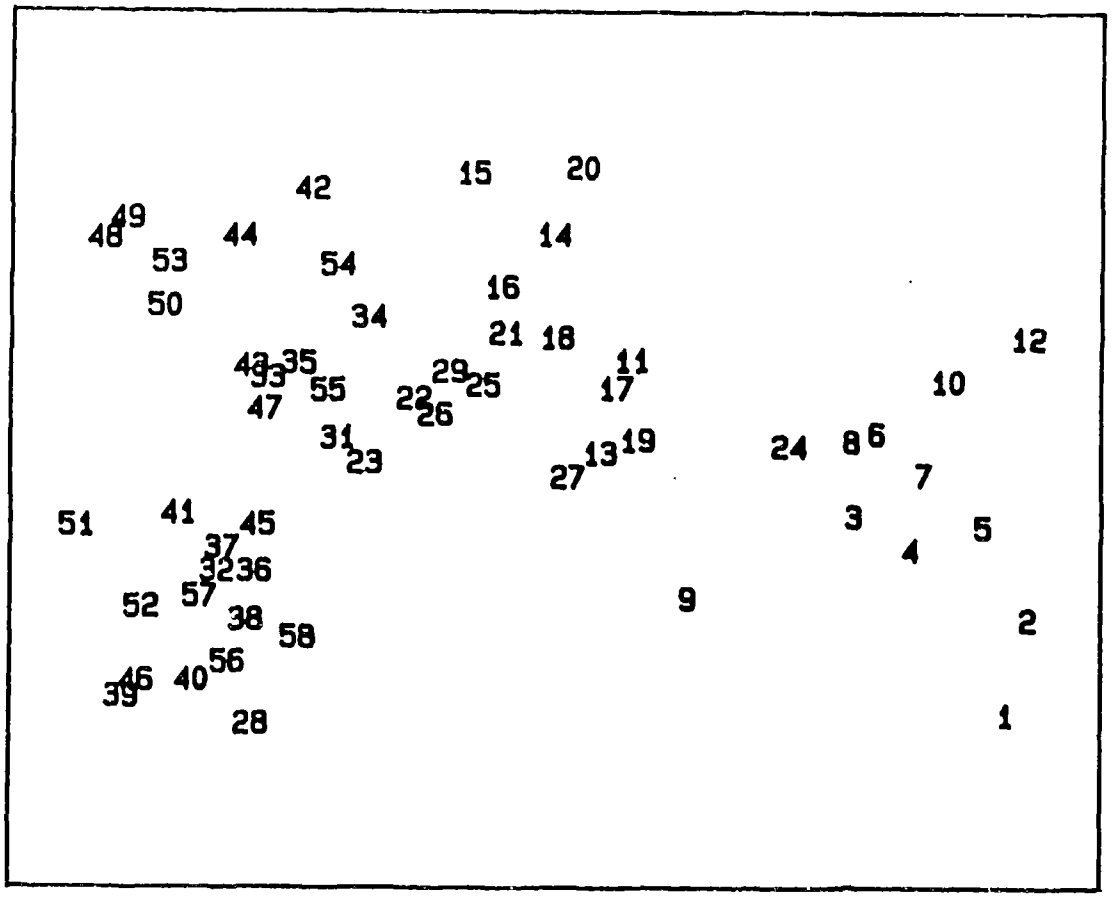


Fig. 11.3. MDS of 57 sites (from Bray-Curtis similarities, on $\sqrt{\sqrt{}}$-transformed counts; stress = 0.11). For map of sites and corresponding cluster analysis, see Figs. 3.2 and 3.3.

Though clear evidence of clusters (from Fig. 3.3), overall pattern is one of GRADATION of COMMUNITY STRUCTURE across the plot (note characteristic "arching", common for strong gradation).

Physical variable driving the structure is SALINITY s, ranging from 24.6‰ (site 1) to 35.1‰ (site 52). Non-linear TRANSFORMATION needed (36‰→35‰ is a more important change than 26‰→25‰); suggest

$$s^* = a - b.\log(36 - s) \qquad (11.1).$$

Choosing $a = 8.33$, $b = 3$ gives $1 \leq s^* \leq 9$, and can:

CATEGORISE (transformed) SALINITY into (say) 9 groups (s^* to nearest integer), and SUPERIMPOSE on MDS.

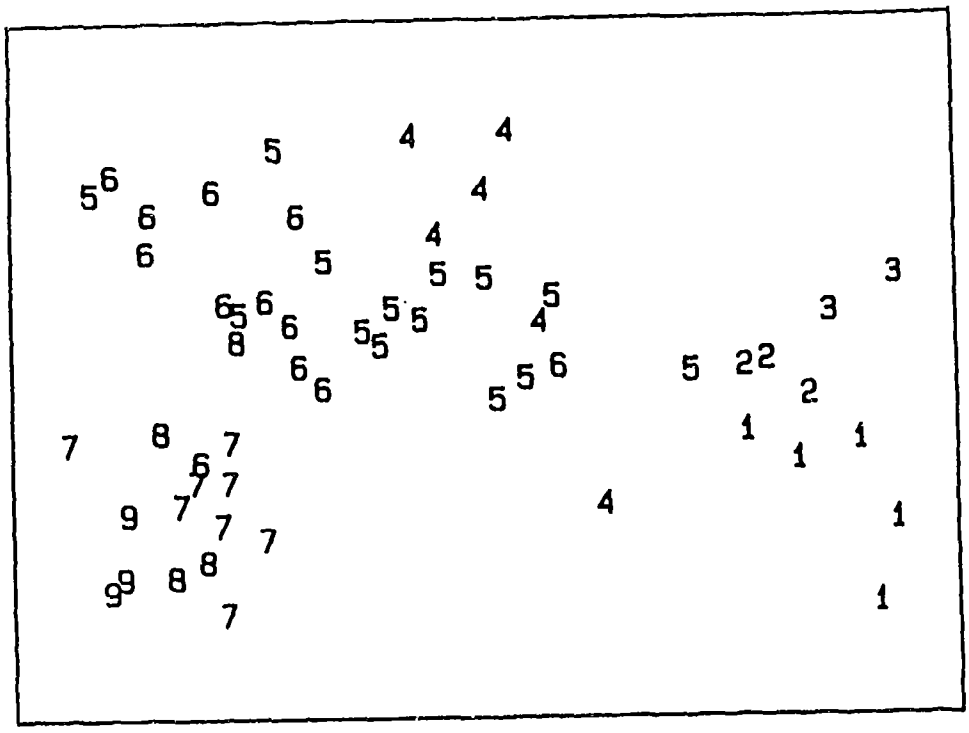


Fig. 11.4. MDS of 57 sites, with increasing salinity categories superimposed. 1: ≤26.3, 2: (26.3, 29.0), 3: (29.0, 31.0), ..., 8: (34.7,35.1), 9: ≥35.1‰.

Alternatively, at each sample point on the faunal MDS, draw a symbol (e.g. circle) with SIZE PROPORTIONAL to the/ ENVIRONMENTAL VARIABLE value for the sample.

Example: Frierfjord macrofauna counts ($\sqrt{\sqrt{}}$-transformed)

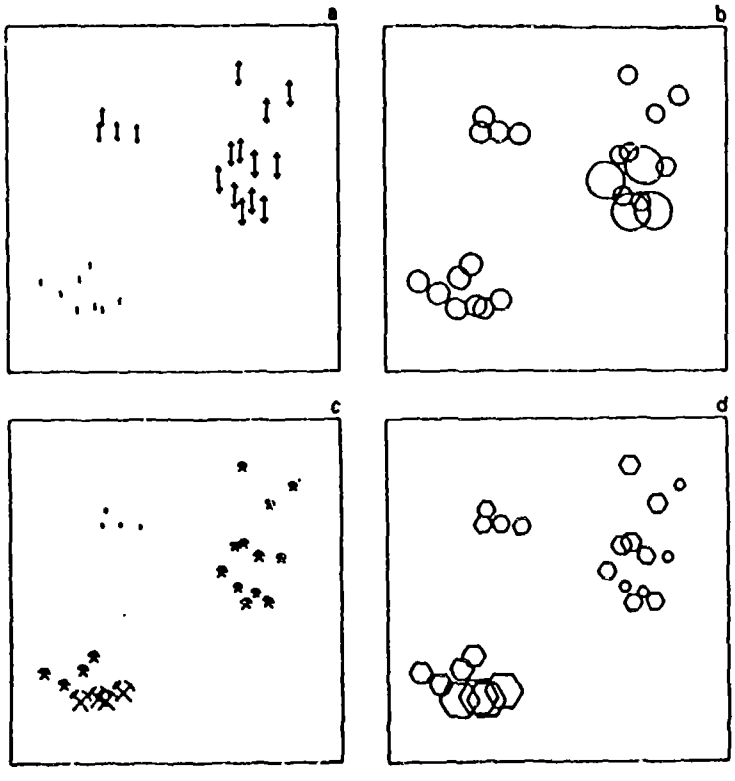


Fig. 11.5. MDS of sites A-E,G with superimposed values of (a) water depth (22-113m), (b) sediment median grain size (7.8-16.5μm), (c) metal levels (PC1 in Fig. 11.1) and (d) "total" PAH (4.4-14.8μg/g).

1) Site grouping on the MDS bears LITTLE RELATION to the (weak) metal and PAH CONTAMINANT GRADIENTS.

2) Sediment granulometry is NOT A DETERMINANT of COMMUNITY DIFFERENCES here (B & C span the range of grain sizes but have the same communities).

3) DEPTH-RELATED differences between the sites appear to be the major CORRELATE of COMMUNITY DIFFERENCES. (Seasonal anoxia in the deeper parts of the fjord is likely to be a signficant "stress" factor.)

Sometimes MORE THAN ONE AXIS OF CHANGE MAY BE SEEN, correlating with different environmental variables.

Example: Exe nematode abundances, Field et al. (1982).

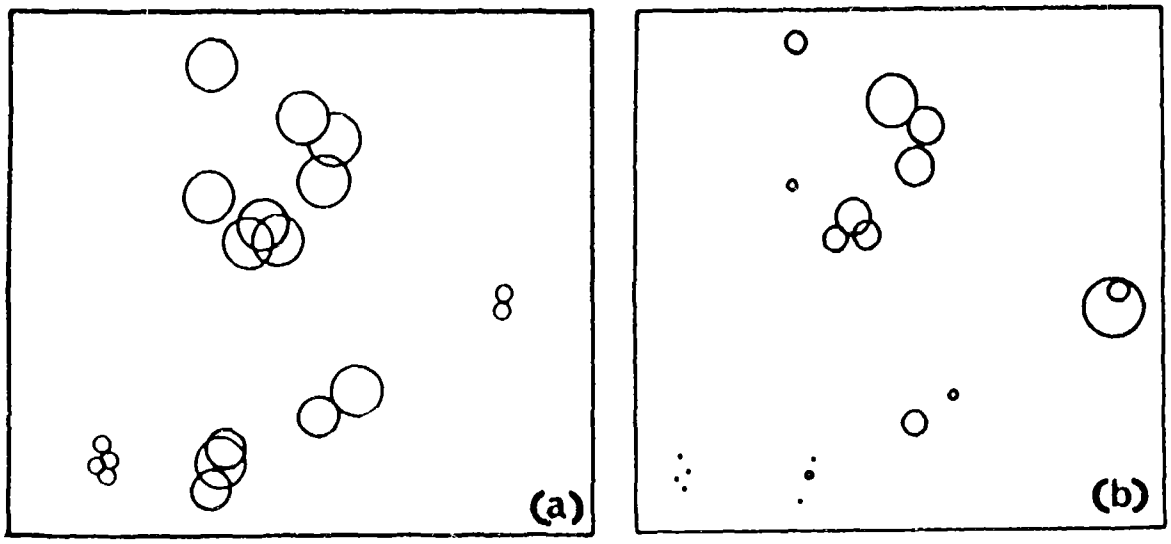


Fig. 11.6. MDS of 19 sites (Fig. 5.1), with values of:

(a) mean salinity of interstitial water (10-90% of standard seawater),
(b) median sediment particle size (0.06-1.14mm), superimposed at each site.

Grain size forms a gradient from bottom left to top right, whereas salinity distinguishes the "middle" from the "end" sites along the first MDS axis.

Though the visual approach is generally more helpful, FORMAL TESTING of gradients can be performed by:

a) REGRESSING each environmental variable on the (x,y) CO-ORDINATES of the SAMPLE LOCATIONS on the MDS; this would be multiple linear regression (and not appropriate for a curvilinear gradient).

b) Using 2-WAY ANOSIM on sites (treated as replicates), which are categorised by, say, 2 environmental variables at 2 levels, e.g. deep/shallow, high/low contaminant loads. This would need a reasonable number of sites (with some in all 4 combinations).

RELATION TO FAUNAL ANALYSES - SECOND APPROACH

First approach designed mainly to show COMMUNITY pattern related to ONE ENVIRONMENTAL VARIABLE at a time. Alternative considers ALL environmental variables together and COMPARES ordination of biota to ORDINATION of environmental variables.

Example: Exe nematode abundances.

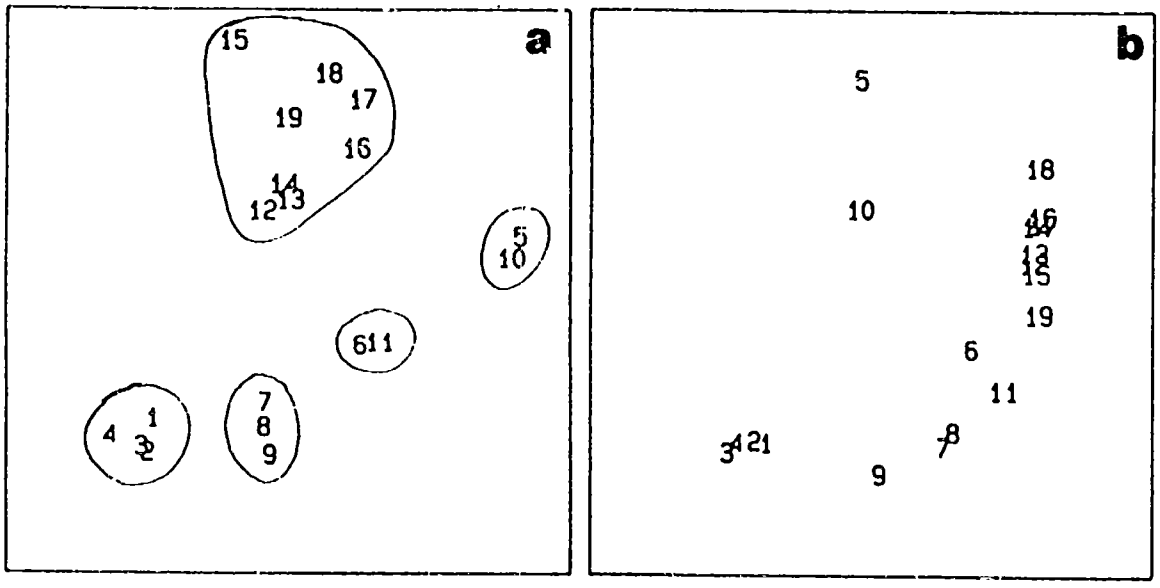


Fig. 11.7. (a) MDS of 19 sites (as in Fig. 5.1),
(b) PCA of 4 environmental variables (salinity, median particle size, % organics, depth of H_2S layer).

The close match of patterns shows these 4 variables "EXPLAIN" biota clusters (in Fig. 11.7a) well.
Two questions: Would subset of environmental variables do as well? Would more variables do better? (e.g. height up shore, water table depth.)

Answer by DEFINING MATCH between two ordinations as some form of RANK CORRELATION (ρ) between underlying DISSIMILARITY MATRICES (Bray-Curtis and Euclidean distance, respectively). Then find subset of environmental variables which MAXIMISES ρ . Here, this is the 4 variables in Fig. 11.7b.

IMPLICATIONS FOR DESIGN

1) SITE SELECTION: where there is choice, attempt to select sites such that VARIATION IN "NUISANCE" (physical) VARIABLES IS SMALL, (i.e. small enough not to have a significant affect on community structure).

2) Where between-site variation in natural variables is considerable, AVOID DESIGNS in which important physical variables are TOTALLY CONFOUNDED (i.e. run in parallel) with contaminant gradients. It may then be possible to DISTINGUISH SEPARATE PHYSICAL AND CONTAMINANT GRADIENTS in an MDS plot.

(Alternatively, choose CONTROL SITES MATCHED to the PHYSICAL VARIABLES for each impacted site.)

3) Where within-site variation in natural variables is considerable (comparable with between-site), MDS distinction of contaminant and natural gradients is greatly AIDED by separate MEASUREMENT of environ- mental variables MATCHING EACH COMMUNITY REPLICATE.

LECTURE 12

CAUSALITY: COMMUNITY EXPERIMENTS IN THE FIELD AND LABORATORY

In experimental situations we can investigate the effects of a single factor (the TREATMENT) on community structure, while other factors are held constant or controlled. There are three main categories of experiments that can be used:

1. 'NATURAL EXPERIMENTS' - Nature provides the treatment: i.e. we compare places or times which differ in the intensity of the environmental factor in question.

2. FIELD EXPERIMENTS - The experimenter provides the treatment: i.e. environmental factors are manipulated in the field.

3. LABORATORY EXPERIMENTS - Environmental factors are manipulated by the experimenter in laboratory mesocosms or microcosms.

The degree of 'naturalness' (hence realism) decreases from 1-3, but the degree of control which can be exerted over confounding environmental variables increases from 1-3.

In all cases care should be taken to avoid PSEUDOREPLICATION, i.e. the treatments should be replicated, rather than a series of 'replicate' samples taken from a single treatment (pseudoreplicates). This is because other confounding variables, often unknown, may also differ between the treatments. It is also important to run experiments long enough for community changes to occur: this favours components of the fauna with short generation times (see Lecture 13).

NATURAL EXPERIMENTS

The obvious logical flaw with this approach is that its validity rests on the assumption that places or times differ only in the intensity of the selected environmental factor (treatment). Experimental design is often a problem, but statistical techniques such as TWO-WAY ANOVA or TWO-WAY ANOSIM, which enable us to examine the treatment effect allowing for differences between sites, are useful.

Example: The effects of disturbance by soldier crabs (Mictyris platycheles) on meiobenthic community structure.

LOCATION: Sand-flat at Eaglehawk Neck, S.E. Tasmania.
SAMPLING: Sediment disturbed by crabs in discrete patches. 4 x 5m^2 blocks of 4 samples with each block including 2 disturbed and 2 undisturbed:

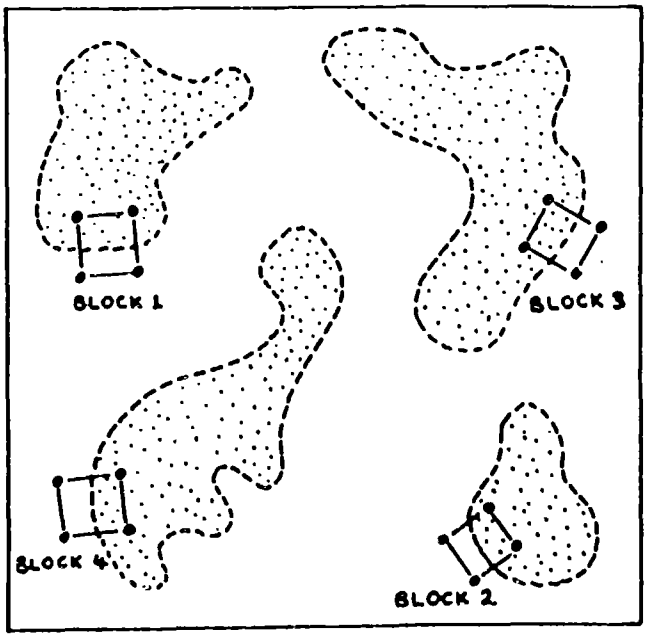


Fig. 12.1. Sketch showing the type of sample design. Sample positions (large dots) in relation to disturbed sediment patches (stippled).

UNIVARIATE INDICES:

TABLE 12.1

Mean values per core sample of univariate measures for nematodes, copepods and total meiofauna (nematodes + copepods) in the disturbed and undisturbed areas. The significance levels for differences are from a two-way ANOVA, i.e. they allow for differences between blocks, although these were not significant at the 5% level.

	Tot.ind.	Tot.sp.	d	H'	J'
Nematodes					
Disturbed	205	14.4	2.6	1.6	0.58
Undisturbed	200	20.1	3.7	2.2	0.74
Significance (%)	91	1	0.3	0.1	1
Copepods					
Disturbed	94	5.4	1.0	0.96	0.59
Undisturbed	146	5.7	1.0	0.84	0.49
Significance (%)	11	52	99	52	38
Total meiofauna					
Disturbed	299	19.8	3.4	2.0	0.66
Undisturbed	346	25.9	4.4	2.3	0.69
Significance (%)	48	1	3	3	16

For NEMATODES: significant reduction in total number of species, Species Richness, Shannon Diversity and Evenness in relation to disturbance.

For COPEPODS: no differences in any of these univariate measures.

GRAPHICAL/DISTRIBUTIONAL PLOTS

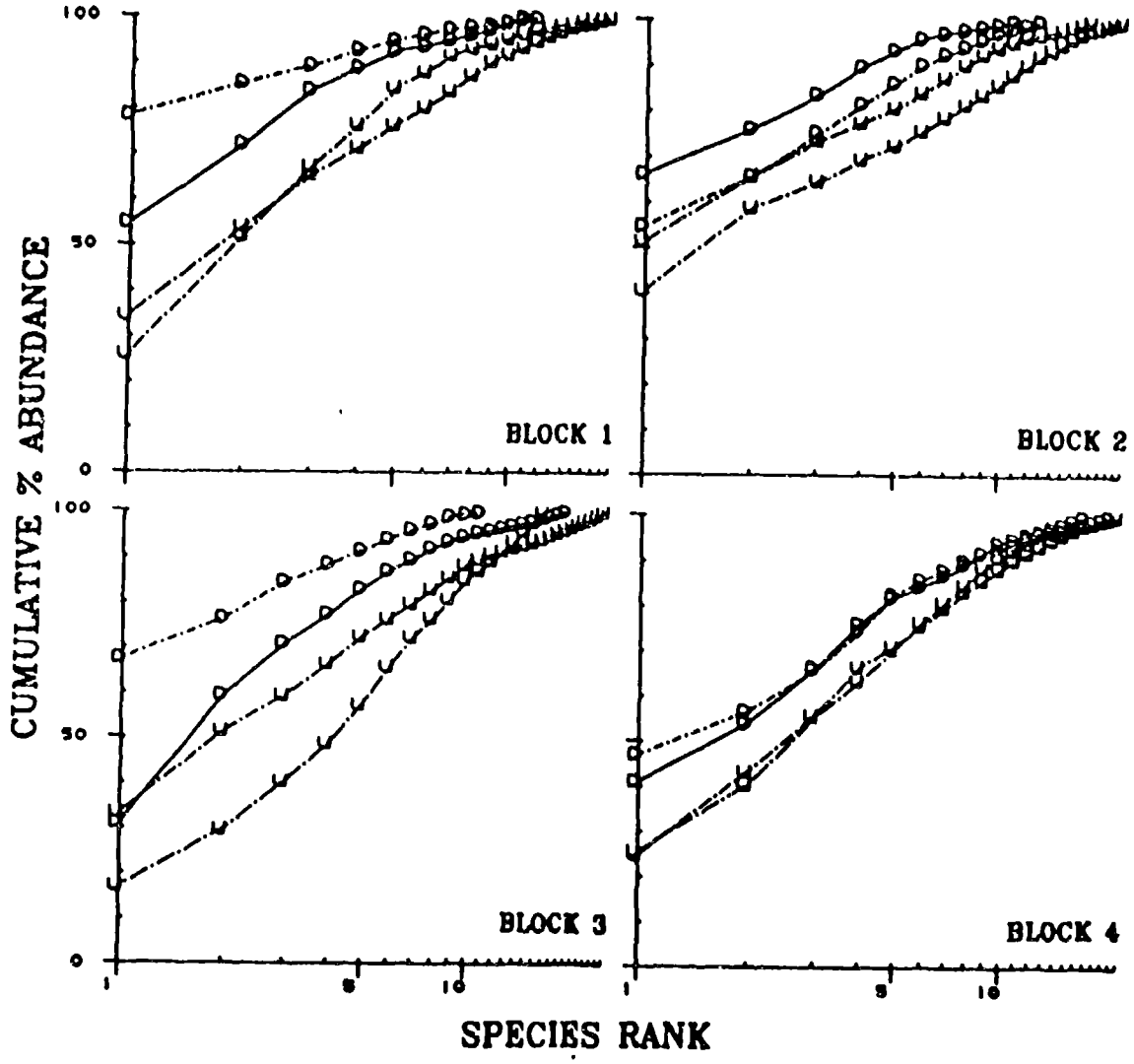


Fig. 12.2. Replicate k-dominance curves for NEMATODE abundance in each sampling block. D = disturbed, U = undisturbed.

Summary statistics K_A and R (see Lecture 8) both show significant treatment effect when tested with two-way ANOSIM.

For COPEPODS (figure not given here), k-dominance curves are intermingled and crossing, and there is no significant treatment effect on K_A and R.

MULTIVARIATE ANALYSIS:

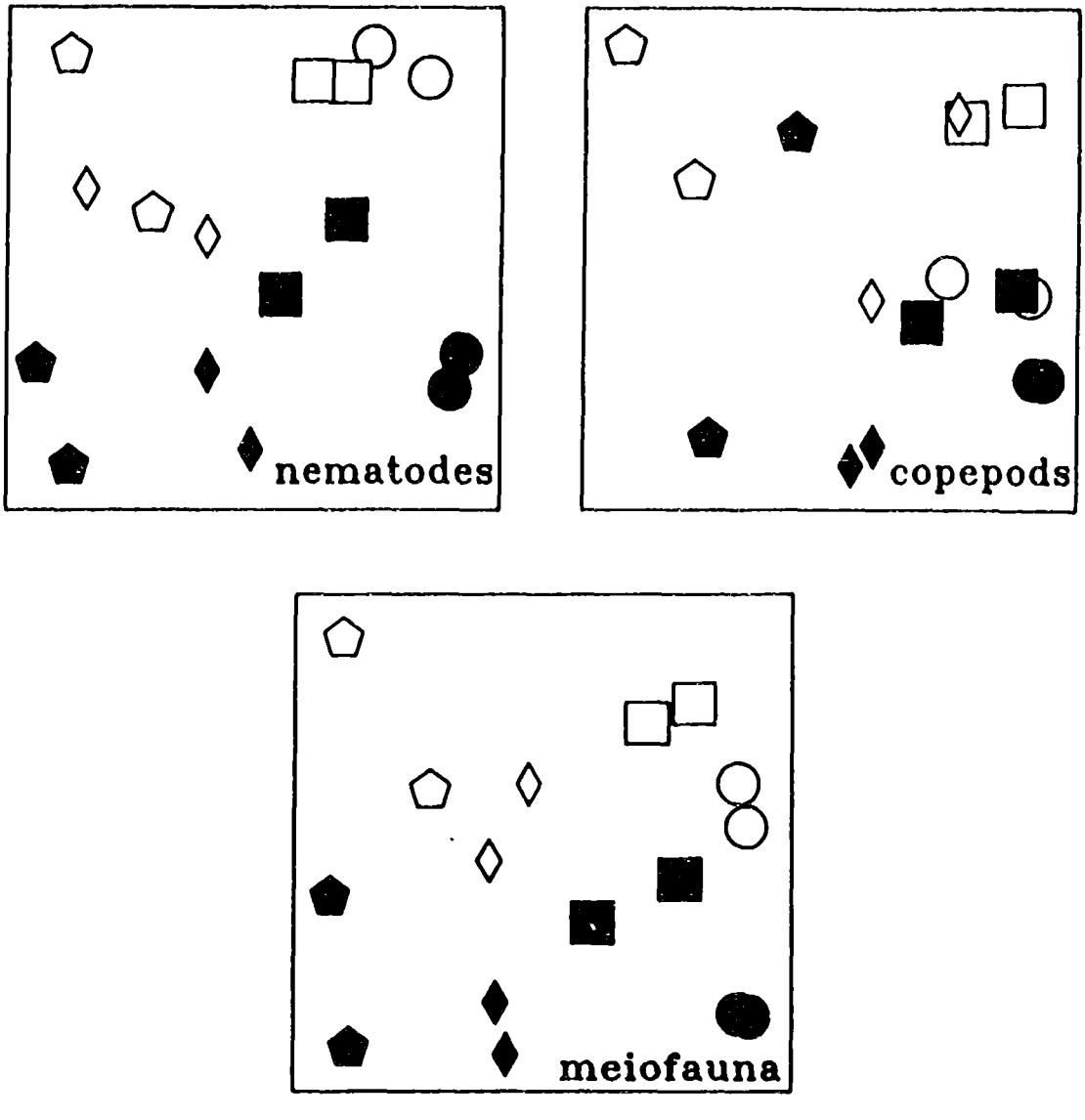


Fig. 12.3. MDS configurations for nematode, copepod and 'meiofauna' (nematode + copepod) abundance. Circles = Block 1, Squares 2, Pentagons 3, Diamonds 4. Open symbols = disturbed, shaded = undisturbed.

Note similarities: both disturbed samples within each block are above both undisturbed; blocks arranged in sequence (left to right) 3,4,2,1.

TABLE 12.2

Results of the two-way ANOSIM test for treatment (disturbance/no disturbance) and block effects.

	DISTURBANCE		BLOCKS	
	R statistic	Sig.(%)	R statistic	Sig.(%)
Nematodes	1.0	1.2	0.99	0.2
Copepods	0.56	3.7	0.70	0.2
Meiofauna	0.94	1.2	0.94	0.2

For both nematodes and copepods, two-way ANOSIM shows significant effect of both treatment (disturbance) and blocks, but differences more marked for nematodes (higher values of R statistic).

CONCLUSIONS:

Univariate indices and graphical/distributional plots only significantly affected by crab disturbance for nematodes. Multivariate analysis reveals similar response for nematodes and copepods (i.e. seems to be more sensitive). In multivariate analyses, natural variations in species composition across the beach (i.e. between blocks) were about as great as those between treatments within blocks: disturbance effect would not have been clearly evidenced without this block sampling design.

FIELD EXPERIMENTS

These include, e.g. caging experiments to exclude or include predators, controlled pollution of experimental plots, big-bag experiments with plankton. Have mostly been used so far for population rather than community studies: not possible to find an example where univariate, graphical/distributional and multivariate techniques have all been applied.

Example: Effect of sediment particle diameter on a harpacticoid copepod community (Hockin, 1982).

LOCATION: Sandy estuarine beach, Ythan estuary, Scotland.
SAMPLING: 2 replicates of 4 grades of glass beads deployed in plastic trays in randomised block design at two tide levels. Left in field for 14 wks, with core sample taken every 5 days.

UNIVARIATE INDICES:

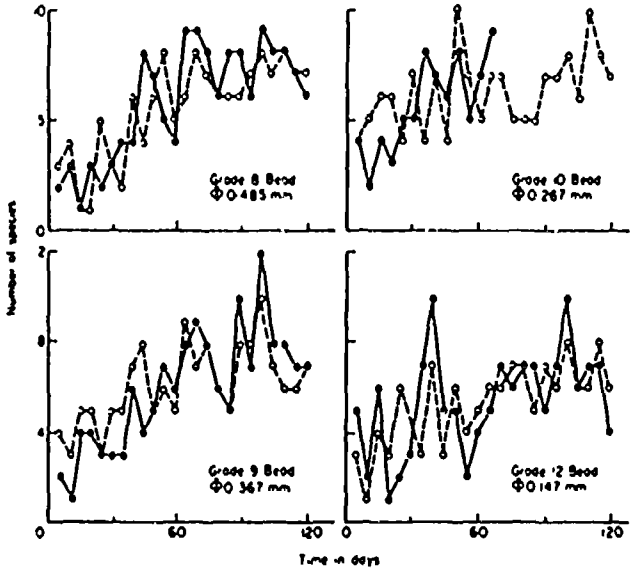


Fig. 12.4 Number of species at upper (solid circles) and lower (open circles) sites.

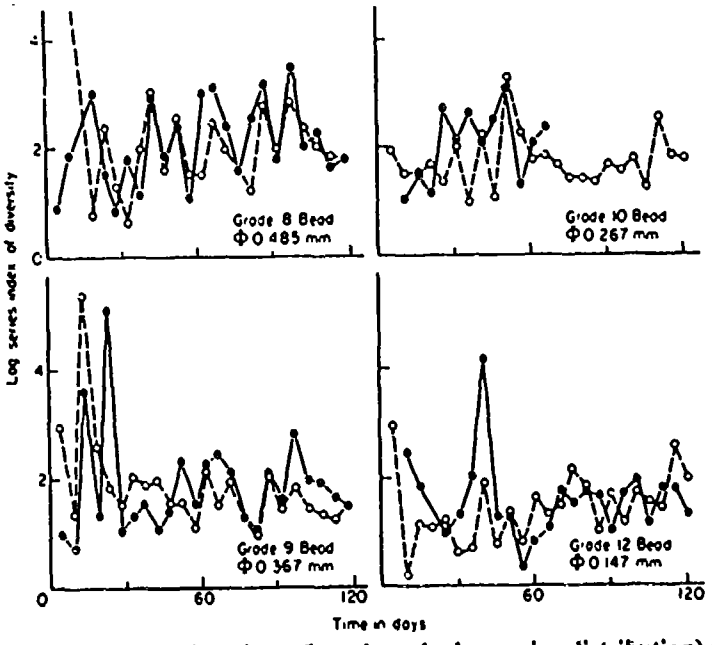


Fig. 12.5. The index of diversity a (based on the log-series distribution) for upper (solid circles) and lower (open circles) sites.

ANOVA on both the number of species and the species diversity revealed no significant differences with respect to the treatment (sediment particle size).

TABLE 12.3

Particle diameter of artificial monometric sediments in which the maximum population densities of the numerically dominant harpacticoid copepod species were found.

COPEPOD SPECIES	PARTICLE DIAMETER (MM)
Arenosetella germanica	0.267
Arenosetella tenuissima	0.367
Arenopontia subterranea	0.147
Evansula incerta	0.367
Stenocaris pygmea	0.267
Heterolaophonte minuta	0.485
Heterolaophonte littoralis	0.485
Esola typhlops	0.367
Paronychocamptus curticaudatus	0.485
Huntemannia jadensis	0.147
Nannopus palustris	0.147

Although no MULTIVARIATE ANALYSES were done, different species reached maximum abundance in different sediment grades. This suggests that a multivariate analysis may well have provided discrimination between treatments.

LABORATORY EXPERIMENTS

More or less natural communities of some components of the biota can be maintained in laboratory mesocosms or microcosms (also in outdoor mesocosms), and subjected to a variety of manipulations.

Example: Effects of organic enrichment on meiofaunal community structure (Gee et al., 1985).

LOCATION: Sediment from Oslofjord; mesocosm at Solbergstrand, Norway.
SAMPLING: Undisturbed 0.25m^2 box cores of sediment transferred to mesocom basin. 4 replicate boxes dosed with high (200 g C.m^{-2}) and low (50 g C.m^{-2}) levels of powdered algae (Ascophyllum), with 4 undosed controls, in randomised block design. Meiofauna sampled 56 days after dosing: 5 cores from each box combined to give one sample.

UNIVARIATE INDICES:

Nematodes: No significant differences in species richness or diversity between treatments, but evenness significantly higher in enriched boxes than controls.

Copepods: Significant differences in species richness and evenness between treatments, but not in diversity.

TABLE 12.4

Univariate measures for all replicates at end of experiment, with F-ratio and significance levels from one-way ANOVA

Treatment	Sample number	Species richness	Shannon-Wiener index	Species evenness
Nematodes				
Control	1	3.023	2.245	0.750
	2	3.739	2.394	0.774
	3	3.357	2.470	0.824
	4	4.589	2.764	0.829
	Total	6.342	2.738	0.747
Low dose	1	4.386	2.856	0 877
	2	2.652	2.474	0.840
	3	4.669	2.885	0.875
	4	2.327	2 268	0.860
	Total	6.153	2.877	0 791
High dose	1	2.856	2 168	0.782
	2	2.824	2.388	0.843
	3	4.302	2.395	0.829
	4	4.088	2 466	0.853
	Total	5.508	2.677	0.759
F-ratio		0.043	1.387	5.131
Significance		ns	ns	$P < 0.05$
Copepods				
Control	1	2.525	1.927	0.927
	2	1.924	1.560	0.969
	3	2.502	1.768	0.908
	4	2.471	1.936	0.931
	Total	2.531	2.102	0.877
Low dose	1	1.804	1.597	0.643
	2	1.661	1.275	0.532
	3	1.655	1.160	0.484
	4	1.786	1.535	0.640
	Total	1.907	1.581	0.584
High dose	1	1.747	1.594	0.767
	2	0.973	0.997	0.620
	3	1.034	0.297	0.165
	4	1.179	1.696	0.872
	Total	1.666	1.683	0 702
F-ratio		17.715	2.654	4.559
Significance		$P < 0.001$	ns	$P < 0.05$

GRAPHICAL/DISTRIBUTIONAL PLOTS:

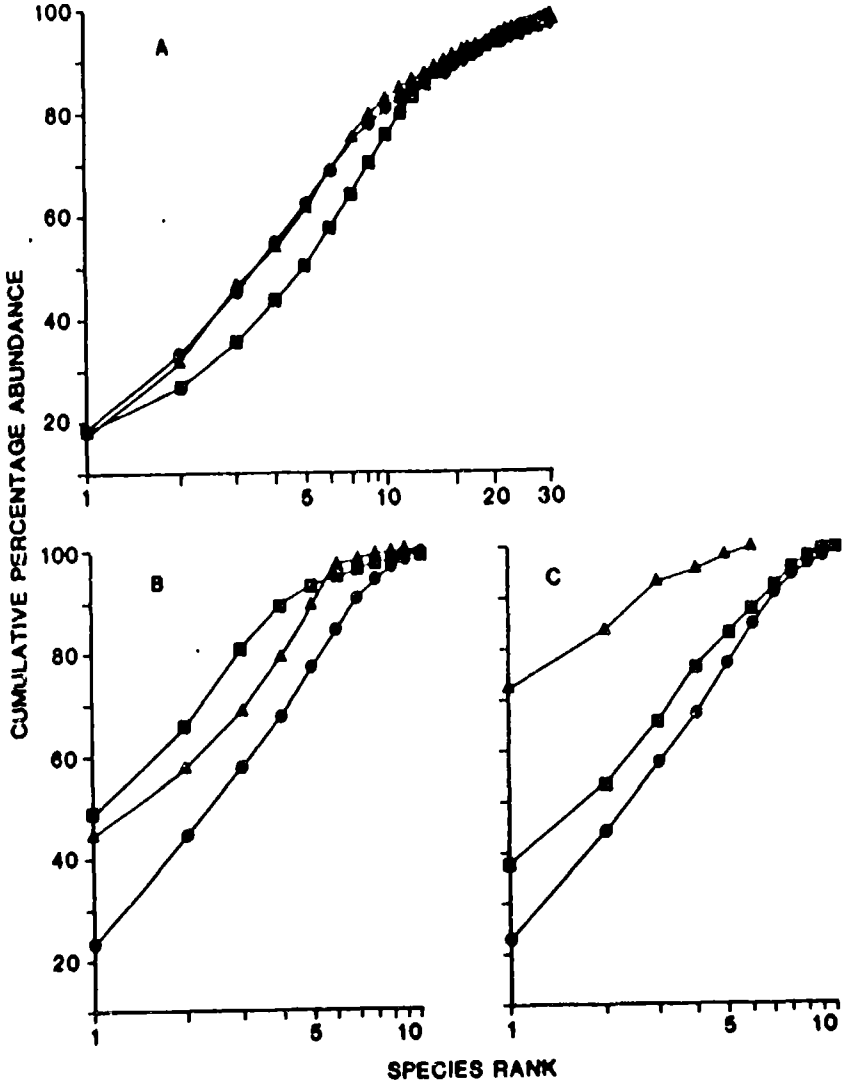


Fig. 12.6. k-dominance curves for A nematodes, B total copepods and C copepods omitting the 'weed' species of Tisbe for summed replicates of each treatment. Circles = control, squares = low dose, triangles = high dose.

NEMATODES: No obvious treatment effect.

COPEPODS: Control with highest diversity; when Tisbe spp. omitted, sequence of increasing elevation of curves (decreasing diversity) from control to high dose.

MULTIVARIATE ANALYSES:

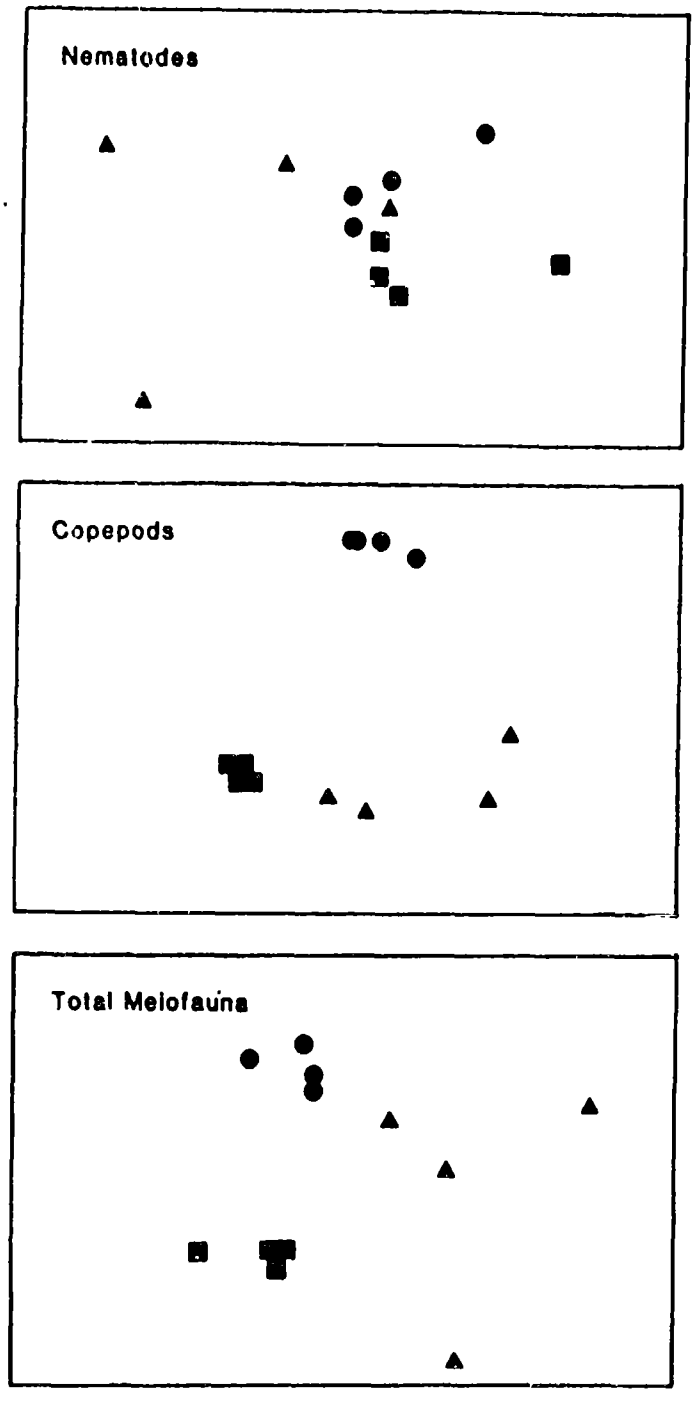


Fig. 12.7. MDS of double square root transformed abundances of nematodes, copepods and total meiofauna (nematodes + copepods). Circles = control, squares = low dose, triangles = high dose.

TABLE 12.5.

Values of the R statistic from the ANOSIM test, in pairwise comparisons between treatments, together with significance levels. C = control, L = low dose, H = high dose.

TREATMENT	STATISTIC VALUE	% SIG LEVEL
Nematodes		
(L, C)	0.27	2.86
(H, C)	0.22	5.71
(H, L)	0.28	8.57
Copepods		
(L, C)	1.00	2.86
(H, C)	0.97	2.86
(H, L)	0.59	2.86

NEMATODES: Only differences between low dose and control treatments are significant at the 5% level.

COPEPODS: Differences between all treatments significant at the 5% level.

Note higher values of the R statistic for copepods in all cases.

CONCLUSIONS: Univariate and graphical/distributional techniques show lowered diversity with increasing dose for copepods, but no effect on nematodes. Multivariate techniques clearly discriminate between treatments for copepods, and still have some discriminating power for nematodes. Changes in nematode community may not have been detectable because of great variability in abundance of nematodes in the high dose boxes.

LECTURE 13

DATA REQUIREMENTS FOR BIOLOGICAL EFFECTS STUDIES: WHICH COMPONENTS AND ATTRIBUTES OF THE BIOTA TO EXAMINE

COMPONENTS:	Pelagos	-	plankton
		-	fish
	Benthos	-	soft-bottom
			- macrobenthos
			- meiobenthos
			- (microbenthos)
			hard-bottom
			- epifauna
			- motile fauna
			- macrofauna
			- meiofauna
ATTRIBUTES:	Abundance	-	species
		-	higher taxa
	Biomass	-	species
		-	higher taxa
	(Production)		

PLANKTON

ADVANTAGES:

- Integrate ecological conditions over areas; useful in monitoring more global changes.
- Taxonomy moderately easy.

DISADVANTAGES:

- Not useful for monitoring local effects, due to mobility.

Example: Continuous Plankton Recorder Survey of NE Atlantic.

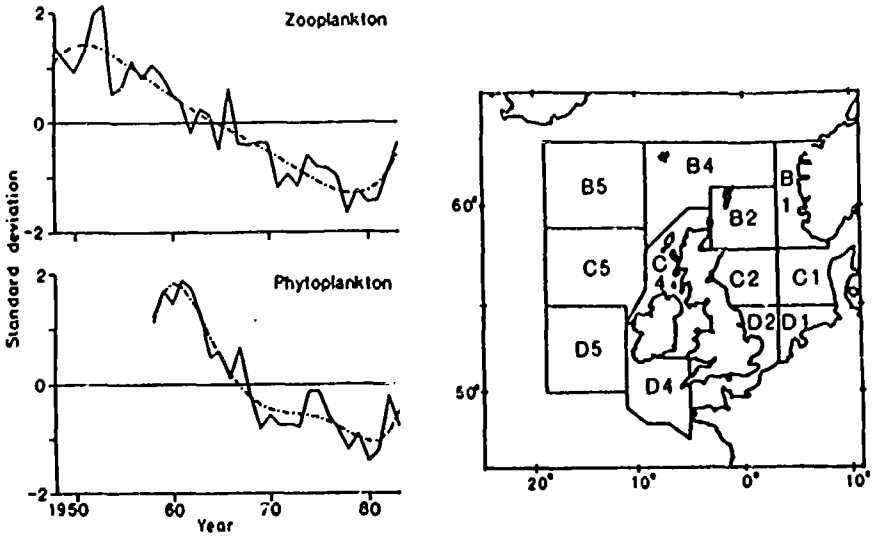


Fig. 13.1. First principal components for zooplankton and phytoplankton (left) in each of the 12 areas shown in the chart (right). Graphs scaled to zero mean and unit variance.

FISH

ADVANTAGES:

- Again more useful for general rather than local effects, but demersal spp. may have site-fidelity
- Taxonomy easy (at least in Europe)
- Of immediate commercial/public interest

DISADVANTAGES:

- Strictly quantitative sampling difficult
- Uncertainty about site-fidelity

Example: Effects of mining activity on coral-reef fish communities in the Maldives.

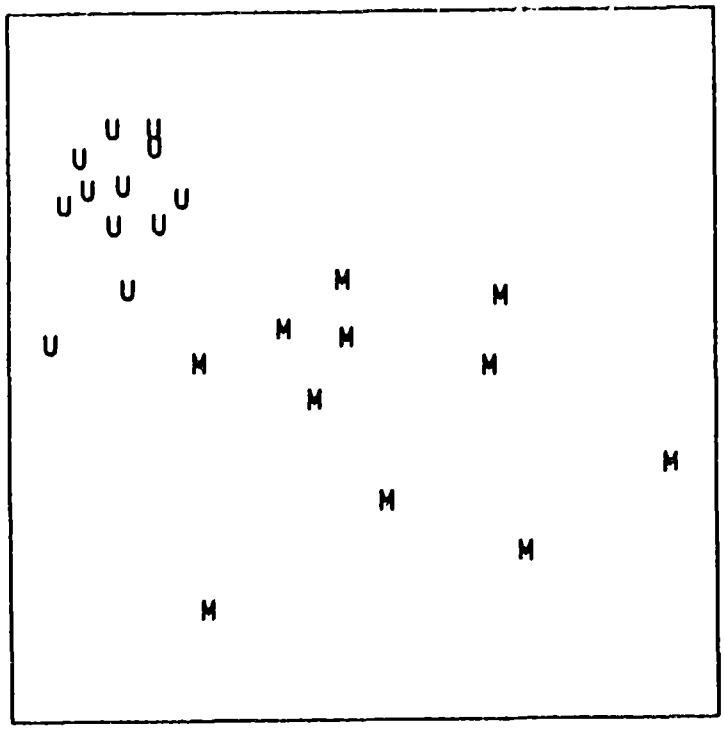


Fig. 13.2. MDS ordination of fish species abundance data from mined (M) and un-mined (U) reef-tops.

MACROBENTHOS

ADVANTAGES:

- Non-mobile, therefore useful for local effects
- Taxonomy relatively easy
- Quantitative sampling easy
- Extensive research literature on community effects

DISADVANTAGES:

- Sampling requires relatively large ships
- Sample-processing at sea labour-intensive
- Response time relatively slow (long generation time)
- Unsuitable for causality experiments (slow response time, planktonic larvae)

Example: Amoco Cadiz oil-spill in the Bay of Morlaix.

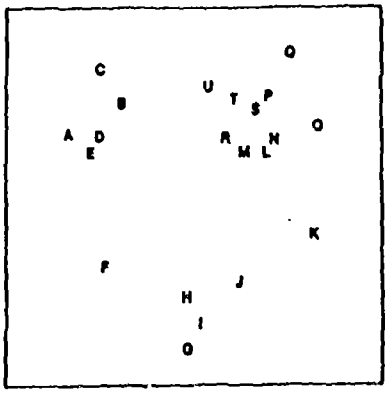


Fig. 13.3. MDS for macrobenthos at station "Pierre Noire". Sampling months are A:4/77, B:8/77, C:9/77, D:12/77, E:2/78, F:4/78, G:8/78, H:11/78, I:2/79, J:5/79, K:7/79, L:10/79, M:2/80, N:4/80, O:8/80, P:10/80, Q:1/81, R:4/81, S:8/81, T:11/81, U:2/82. Oil-spill was during 3/78, i.e. between E and F.

MEIOBENTHOS

ADVANTAGES:

- Useful for local effects studies
- Quantitative sampling easy from small ships
- Samples need not be processed on ship
- Potentially fast response (short generation time)
- Good for causality experiments (direct benthic development, fast response)

DISADVANTAGES:

- Taxonomy considered difficult
- Community responses not well known or documented

Example: Effects of soldier crab disturbance on nematode assemblages at Eaglehawk Neck, Tasmania.

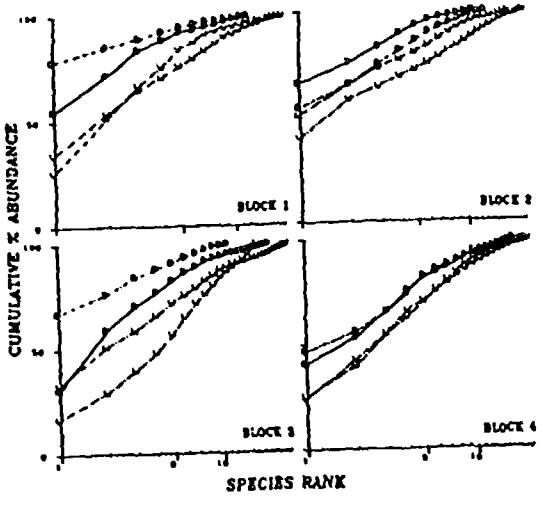


Fig. 13.4. k-dominance curves for disturbed (D) and undisturbed (U) samples in 4 separate sampling blocks.

The macrobenthos & meiobenthos may RESPOND DIFFERENTLY to different kinds of perturbation (e.g. physical disturbance, "pollution") so that a comparative study of both may be indicative of the cause.

Example: Hamilton Harbour, Bermuda.

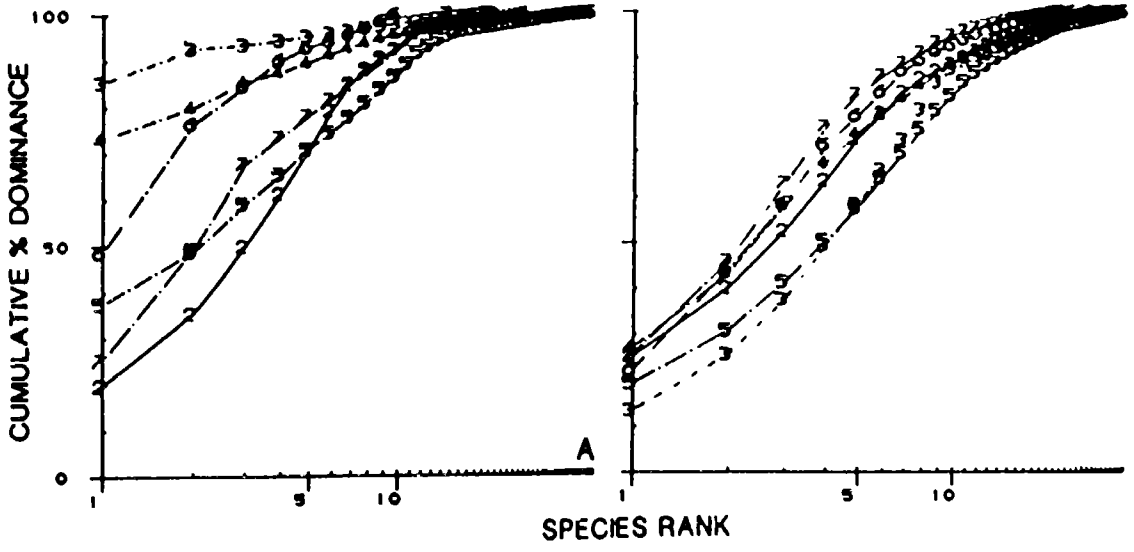


Fig. 13.5. k-dominance curves for macrobenthos (left) and meiobenthic nematodes (right) at six stations in Hamilton Harbour, Bermuda. Elevated macrofauna curves at stations 3 and 4 suggest that physical disturbance is the cause, since the corresonding meiofauna curves at these sites are not similarly affected.

HARD-BOTTOM EPIFAUNA

ADVANTAGES:

- Immobile; good for local effects
- Two dimensional nature permits non-destructive (visual) sampling for determination of temporal changes

DISADVANTAGES:

- Remote sampling difficult
- Enumeration of colonial organisms difficult
- Biomass measurements difficult

Example: Effects of the 1982-3 El Niño on Indonesian reef corals.

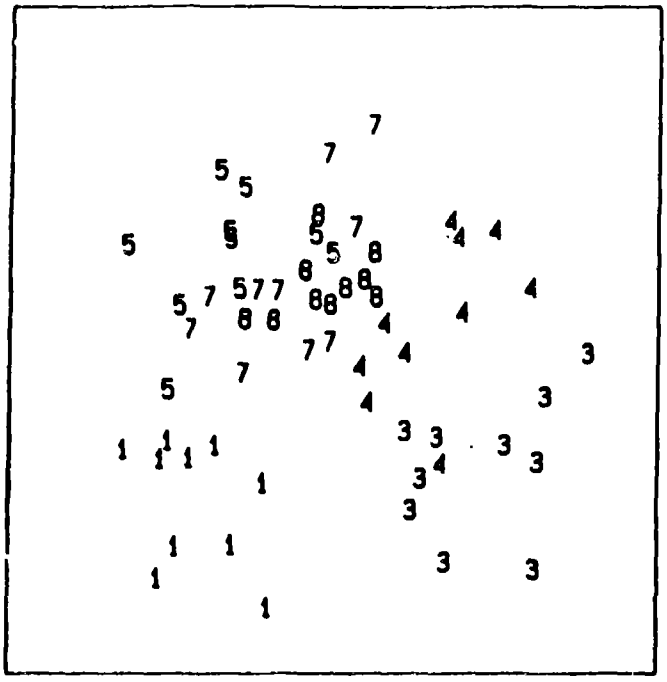


Fig. 13.6. MDS for coral species percentage cover data for South Pari Island. 1=1981, 3=1983 etc.

HARD-BOTTOM MOTILE FAUNA

DISADVANTAGES:

- Remote sampling difficult
- Quantification difficult
- Responses to perturbation not known
- Suitable habitat (e.g. algae) not always available

Example: Macrofauna and meiofauna of replicated intertidal seaweed samples from the Isles of Scilly.

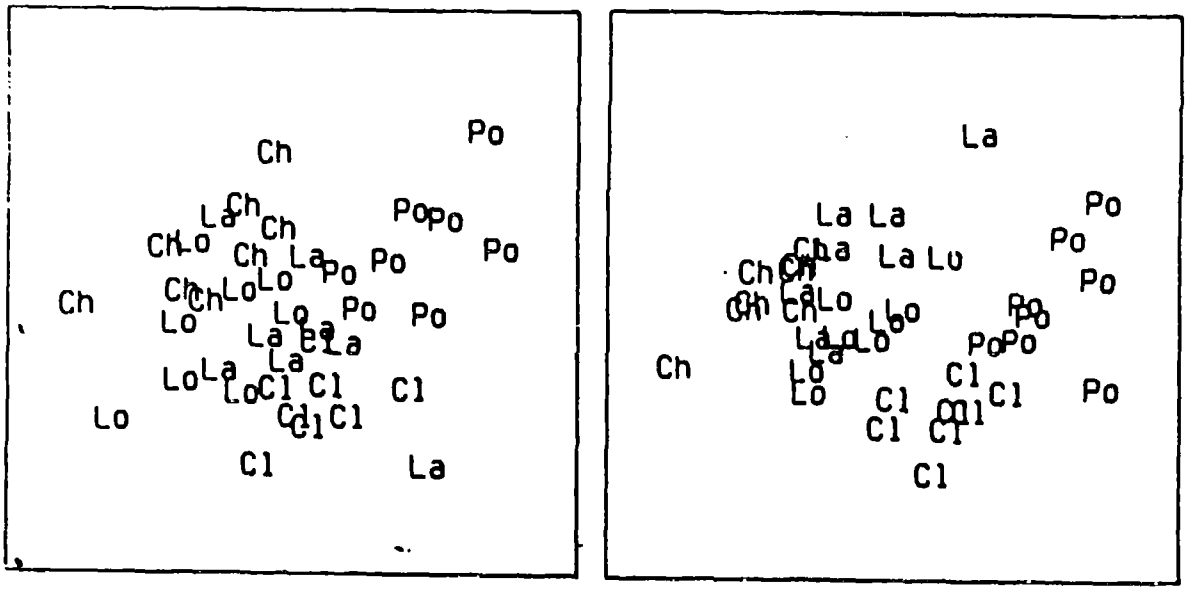


Fig. 13.7. MDS macrobenthos (left) and meiobenthos (right) from different species of seaweeds: Ch=Chondrus, Lo=Lomentaria, La=Laurencia, Cl=Cladophora, Po=Polysiphonia. Note similarity between the two configurations.

ABUNDANCE, BIOMASS OR BOTH?

Abundances are easier to measure, but biomass may be a better reflection of the ecological importance of a species within a community. In practice, multivariate analyses of abundance and biomass data give remarkably similar results, despite the fact that the species mainly responsible for discriminating between stations are different.

Example: Frierfjord macrofauna

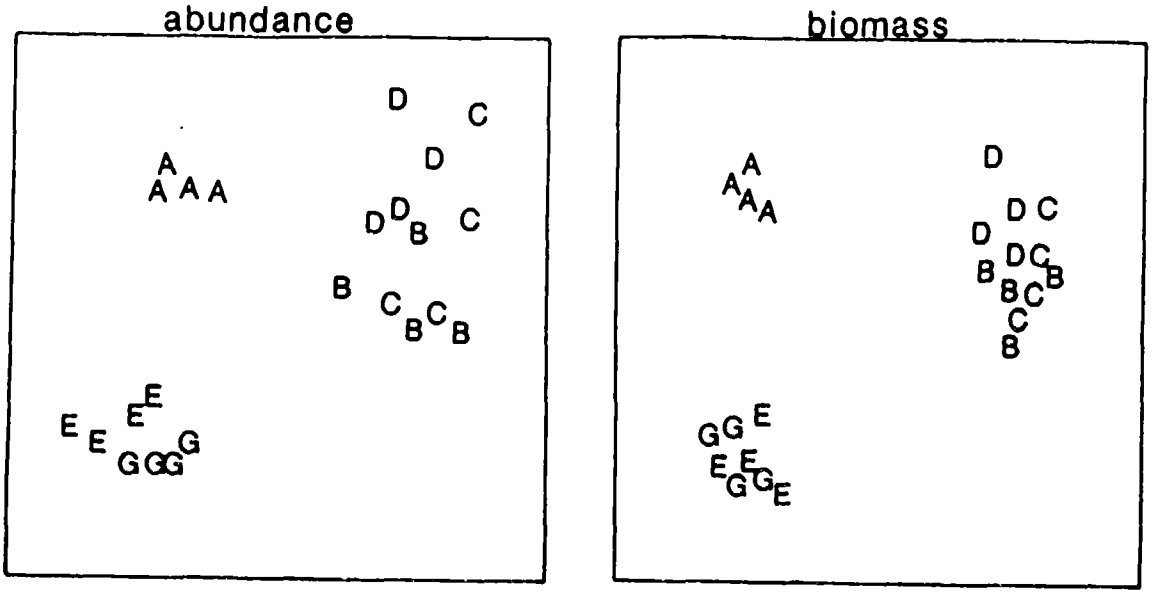


Fig. 13.8. MDS ordinations for macrofauna abundance and biomass. Note the close similarity.

Perturbations of various kinds may affect the distribution of numbers of individuals among species differently from the distribution of biomass among species. This is the basis of the 'ABC' (Abundance Biomass Comparison) method for the assessment of disturbance, which was dealt with in Lecture 8.

SPECIES OR HIGHER TAXA

In a wide variety of pollution-impact studies, it has been found for both graphical-distributional and multivariate analyses that there is surprisingly little loss of information when the species data are aggregated into higher taxa, e.g. genera, families or even phyla. Initial collection of data at the level of higher taxa would result in a considerable saving of time (and cost) in the analysis of samples. This was dealt with in more detail in Lecture 10.

RECOMMENDATIONS

It is difficult to give firm recommendations as to which components or attributes of the biota should be studied, since this depends on the problem in hand and the expertise and funds available. In general, however, the wider the variety of components and attributes studied, the easier the results will be to interpret. A broad approach at the level of higher taxa is often preferable to a painstakingly detailed analysis of species abundances. If only one component of the fauna is to be studied, then consideration should be given to working up a larger number of stations/replicates at the level of higher taxa in preference to a small number of stations at the species level. Of course, a large number of stations at the species level is always the ideal

LECTURE 14

RELATIVE SENSITIVITIES AND MERITS OF UNIVARIATE, GRAPHICAL/DISTRIBUTIONAL AND MULTIVARIATE TECHNIQUES

Two communities with a completely different taxonomic composition may have identical univariate or graphical /distributional structure, and conversely those comprising the same species may have very different univariate or graphical/distributional structure. Do species dependent and species independent attributes of community structure behave the same or differently in response to environmental changes, and which are the most sensitive? These questions will be addressed by reference to a number of case studies in which a variety of methods of data analysis has been employed.

Example 1: Macrobenthos from Frierfjord/ Langesundfjord, Norway (IOC/GEEP Oslo Workshop).

MAP OF SITES: See Fig. 1.1.

UNIVARIATE INDICES:

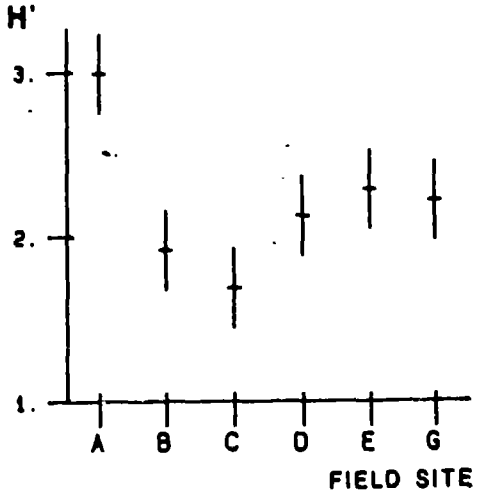


Fig. 14.1. Means (and 95% CIs) for diversity H'.

Site A has higher species diversity (H') and site C the lowest: others not significantly different.

GRAPHICAL/DISTRIBUTIONAL PLOTS:

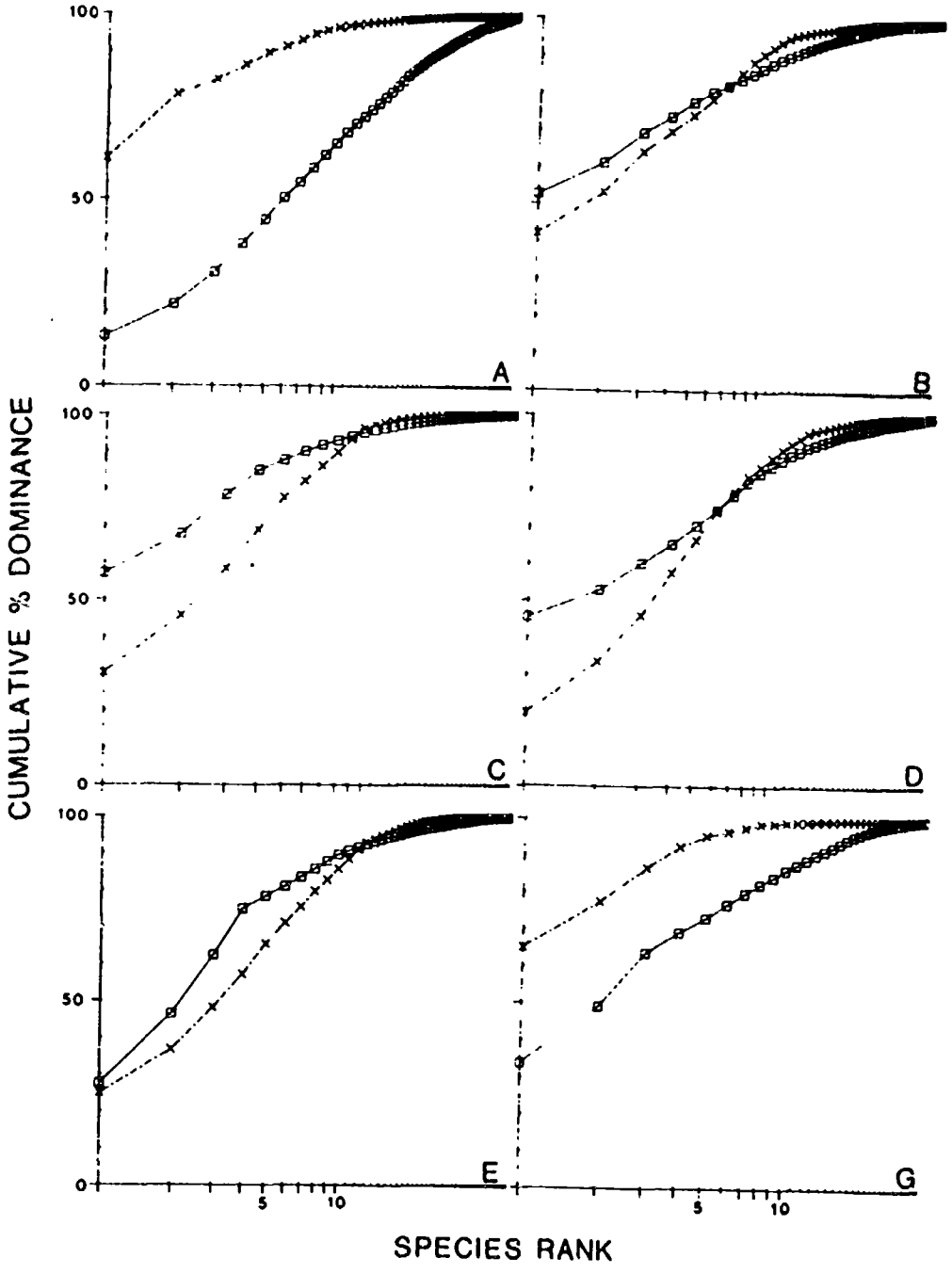


Fig. 14.2. ABC plots based on totals of 4 replicates. Squares = abundance, crosses = biomass.

These indicate C, D and E most stressed, B moderately stressed, A and G unstressed. No tests have been done to determine significance of differences.

MULTIVARIATE ANALYSES:

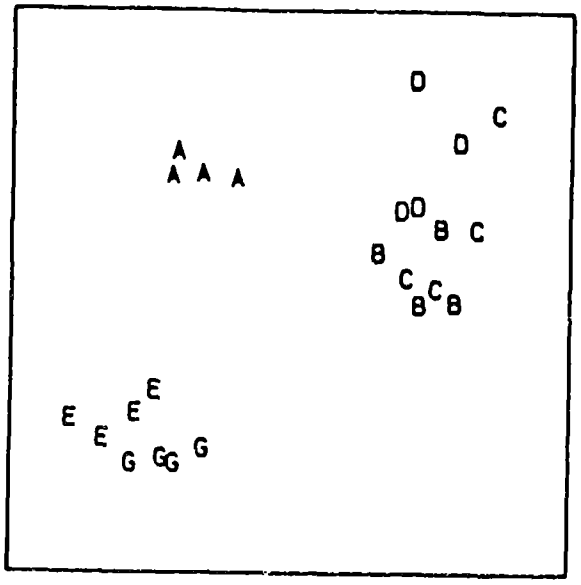


Fig. 14.3. MDS of 4 replicates at each of sites A-E,G (Bray-Curtis similarities on $\sqrt{\sqrt{}}$-transformed counts).

Stations B,C and D cluster together (ANOSIM separates B from A and C) , E and G together (separated with ANOSIM), A on its own. Clusters correlate with water depth rather than measured levels of anthropogenic variables (see Fig. 11.5)

CONCLUSIONS: Multivariate analysis the most sensitive for discriminating stations (only B and C not significantly different). Univariate and graphical distributions conflict with this. For example, E & G have different ABC plots but cluster together; diversity at E is not significantly different from D, but they are the furthest apart on the MDS plots. However, B,C and D all have low diversity and ABC indicates disturbance. Most likely explanation is that these deep-water stations are affected by seasonal anoxia, rather than anthropogenic pollution.

Example 2: Macrobenthos from Hamilton Harbour, Bermuda (IOC/GEEP Bermuda Workshop).

MAP OF SITES:

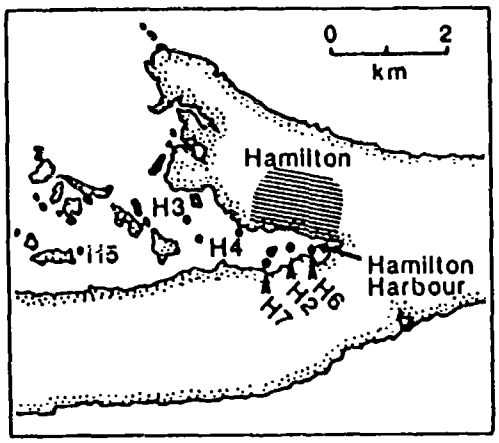


Fig. 14.4. Map of Hamilton Harbour showing locations of 6 sampling stations.

UNIVARIATE INDICES: See Fig. 8.1. H5 with highest diversity, H3 and H4 with lowest diversity (significantly below neutral model prediction, see Table on page 8-4).

GRAPHICAL/DISTRIBUTIONAL PLOTS: ABC curves show H2, H6 and H7 undisturbed, H5 moderately disturbed, H3 and H4 moderately/grossly disturbed (Fig. 14.5)

MULTIVARIATE ANALYSES: On MDS (Fig. 14.6) stations ordered (left to right) 5,4,3,2,7,6. ANOSIM gives all sites significantly different from each other. Superimposing values of environmental variables shows close correlation with metals and TBT, not with water depth, sediment type or hydrocarbons.

CONCLUSIONS: MDS most sensitive in discriminating sites, and relates to pollution levels. Diversity not ordered in the same way. Stations with highest pollution levels not the most 'stressed'.

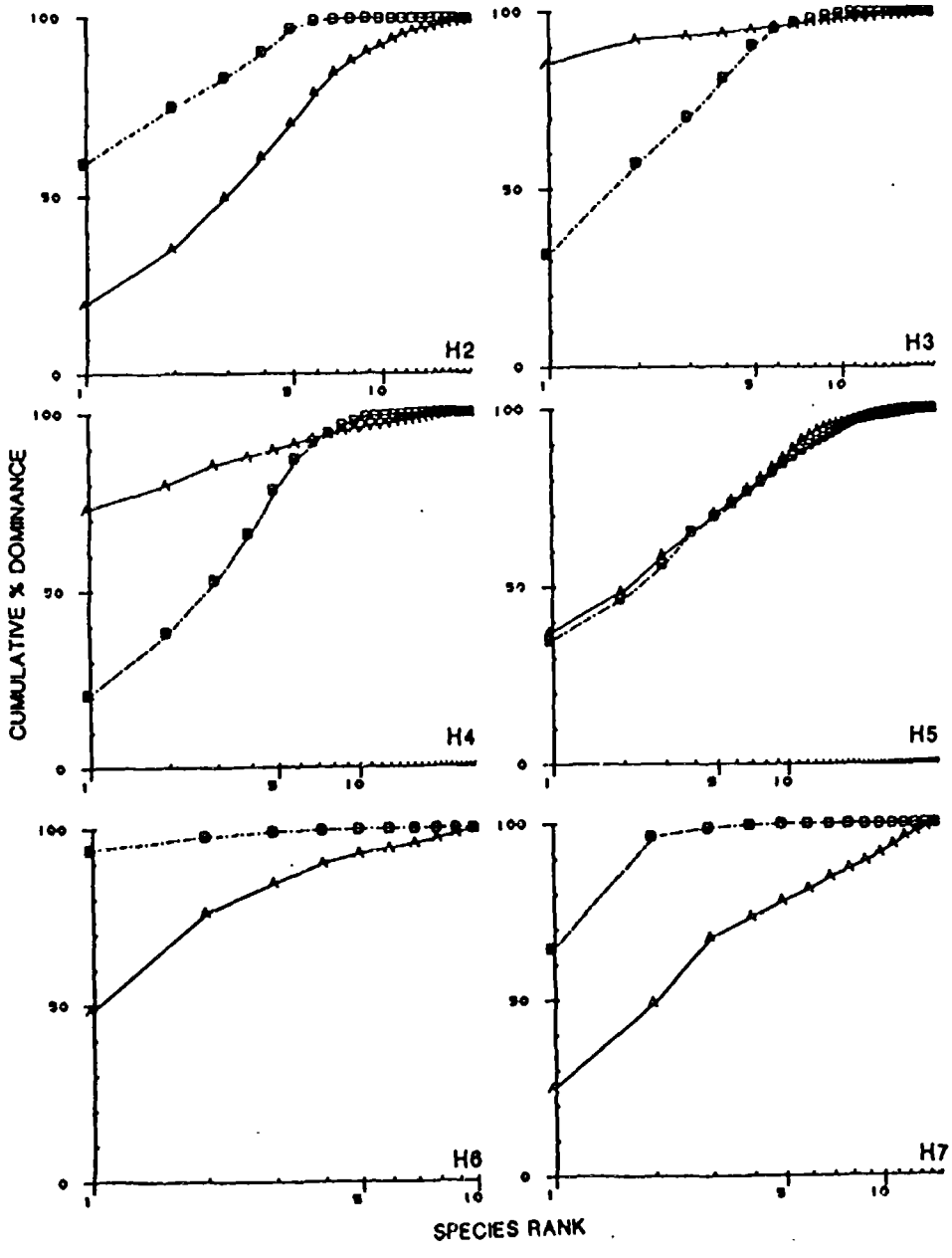


Fig. 14.5. ABC curves for Hamilton Harbour macrobenthos (sum of 4 replicates at each station); A = abundance, B = biomass.

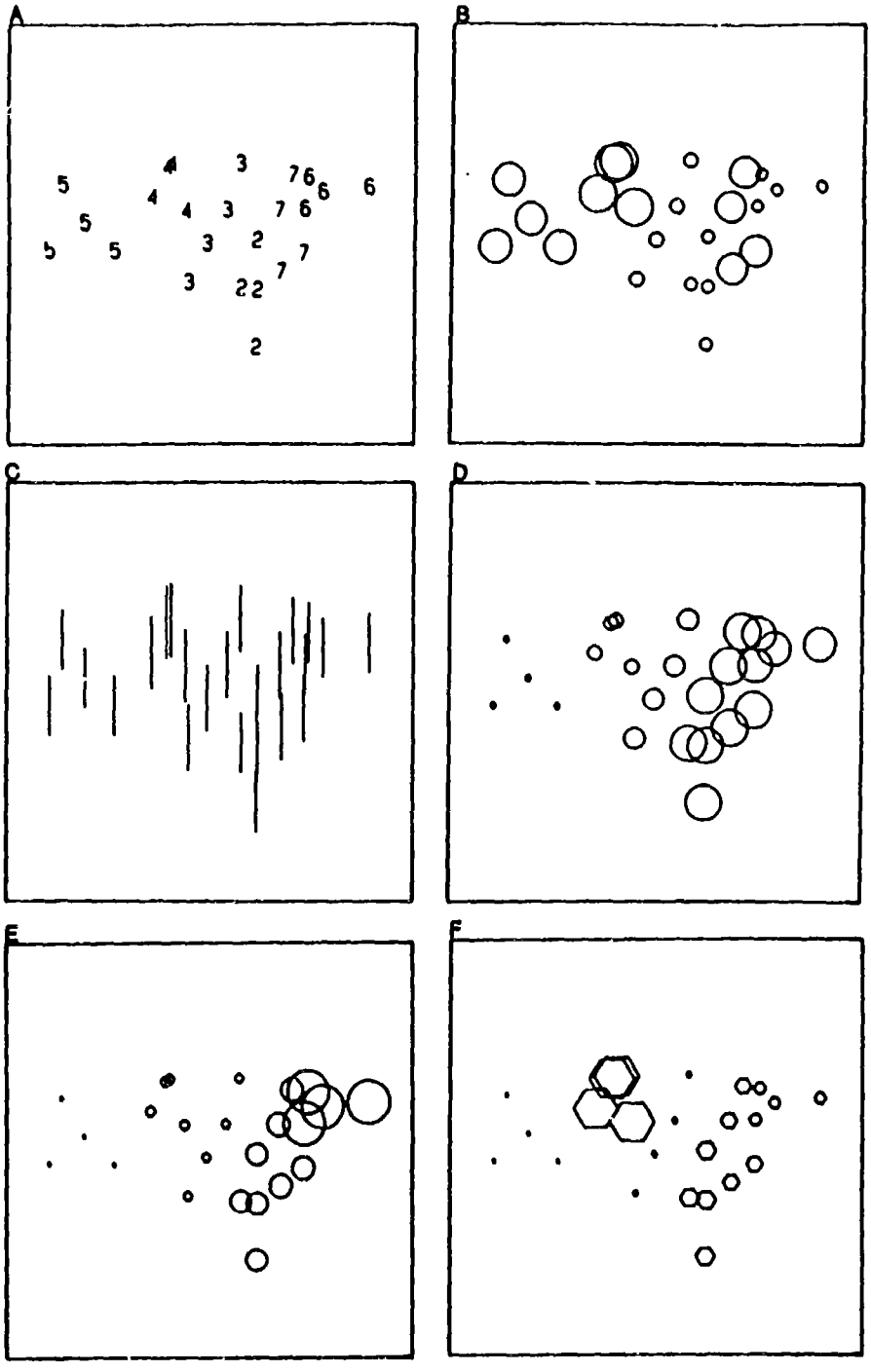


Fig. 14.6. A) 2-D MDS configuration for macrofauna standardised root-transformed abundance. B-F) same configuration with symbols representing values of environmental variables superimposed: B) grain size, C) water depth, D) sediment Pb concentration, E) TBT in water, F) sediment PAH.

Example 3: Reef corals at South Tikus Island, Indonesia, before and after 1982-3 El Niño.

MAP OF SITES: Not available. Ten sets of 3 x 10m transects across reef-flat in each year.

UNIVARIATE INDICES: See Fig. 8.2. Immediate post El Niño decline in number of species and H', slight recovery in 1984 but no significant change after this. No significant changes in J'.

GRAPHICAL DISTRIBUTIONAL PLOTS: From 1984 onwards, k- dominance curves lie entirely above that of 1981, indicating no apparent recovery. With ANOSIM, few significant differences between years detectable after 1984.

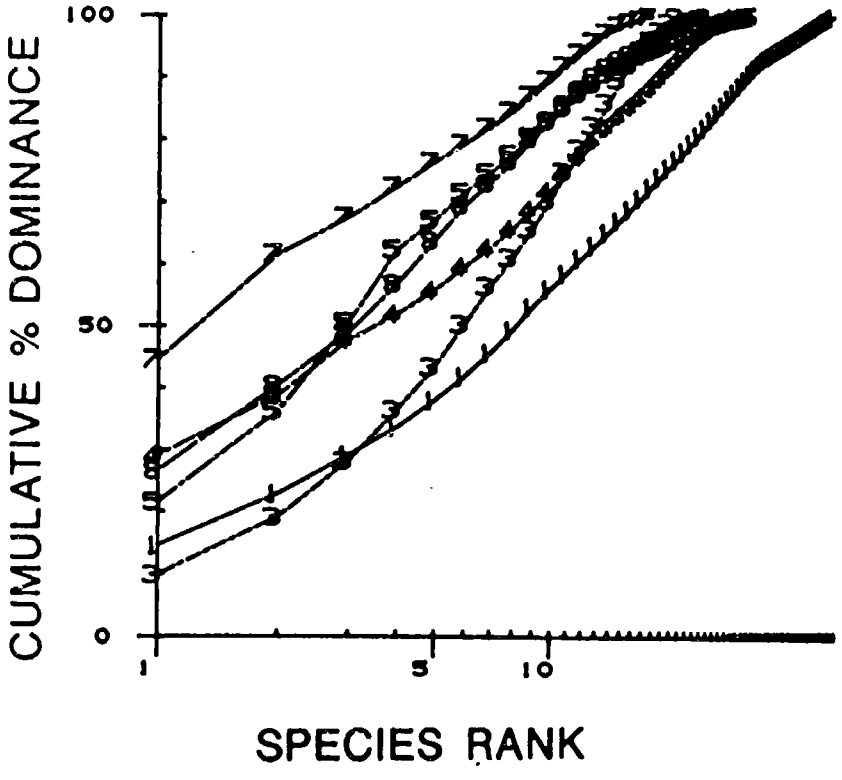


Fig. 14.7. k-dominance curves for totals of all ten replicates in each year. 1=1981, 2=1982 etc.

MULTIVARIATE ANALYSES:

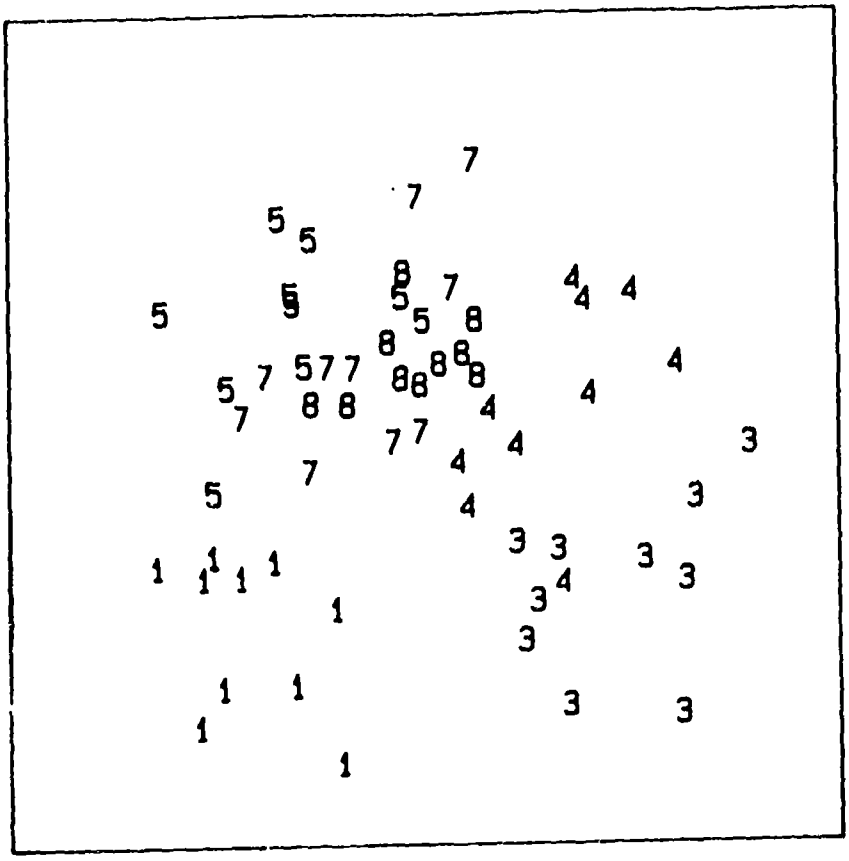


Fig. 14.8. MDS for coral species percentage cover data for South Pari Island. 1 = 1981, 3 = 1983 etc.

El Niño location shift between 1981 and 1983, with gradual recovery towards the 1981 condition until 1985, then a slight move away again in 1987 and 1988. ANOSIM shows all pairs of years to be significantly different.

CONCLUSIONS: All methods demonstrate the dramatic post El Niño decline in species, though the multivariate techniques were seen to be more sensitive in monitoring the recovery phase in later years.

Example 4: Fish communities from mined and non-mined reef tops in the Maldives.

MAP OF SITES: Not available.

UNIVARIATE INDICES: ANOVA shows no significant effect of mining on H' or J'.

GRAPHICAL/DISTRIBUTIONAL PLOTS. k-dominance curves for individual replicates given in Fig. 14.9. ANOSIM shows no significant difference between mined and non-mined sites.

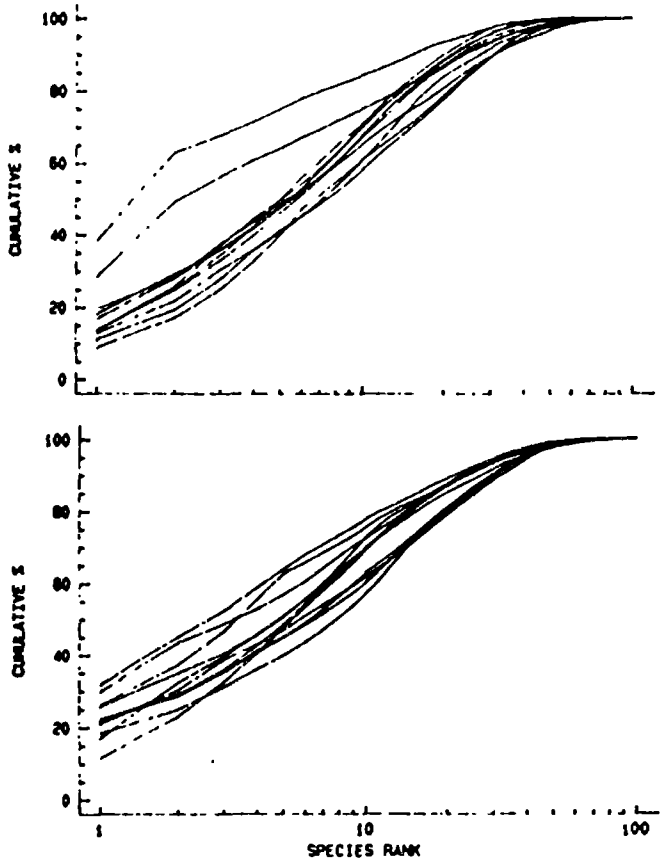


Fig. 14.9. Replicate k-dominance curves for fish communities from mined (top) and non-mined (bottom) reef-tops.

MULTIVARIATE ANALYSES:

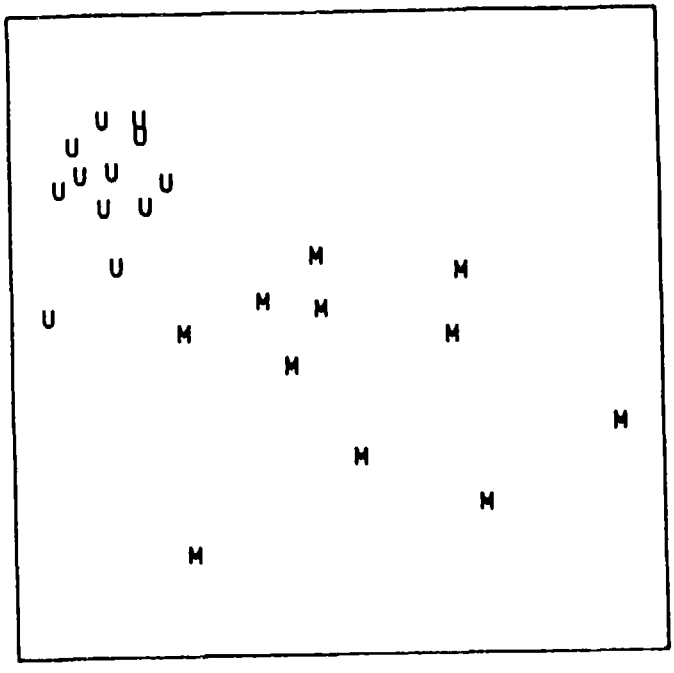


Fig. 14.10. MDS of fish species abundance data from mined (M) and un-mined (U) reef-tops.

Clear separation of mined and non-mined sites, which ANOSIM shows to be significant (though test is unnecessary in such a clear-cut case).

CONCLUSIONS: Clear difference in community composition due to mining activity revealed by multivariate methods, but not detected at all by univariate or graphical/ distributional techniques.

Example 5: Macro- and meiobenthos from different seaweed species on the Isles of Scilly.

MAP OF SITES:

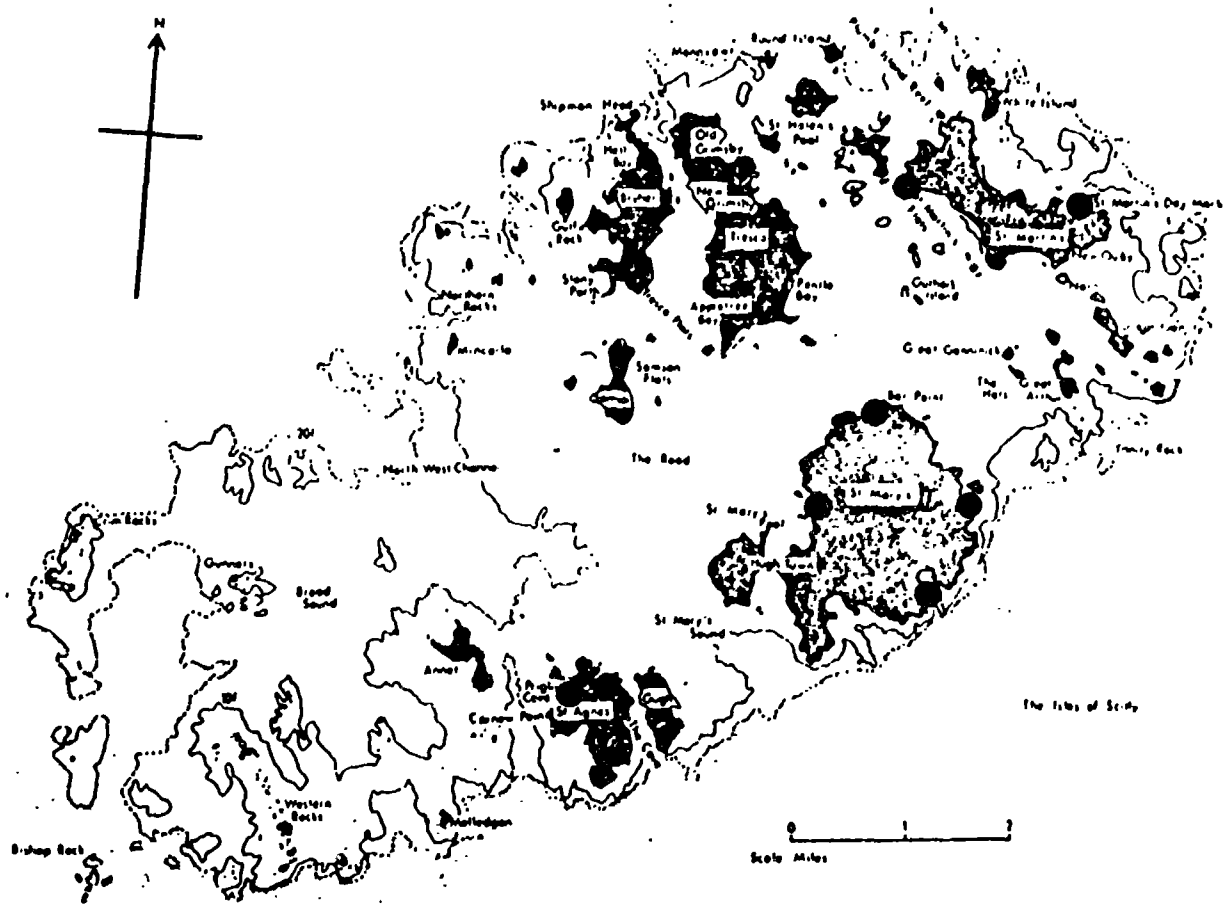


Fig. 14.11. Eight sites on the Isles of Scilly from each of which 5 seaweed species were collected.

UNIVARIATE INDICES: Note that meiobenthos and macrobenthos show different trends, and for all indices many pairs of weeds are not significantly different from each other.

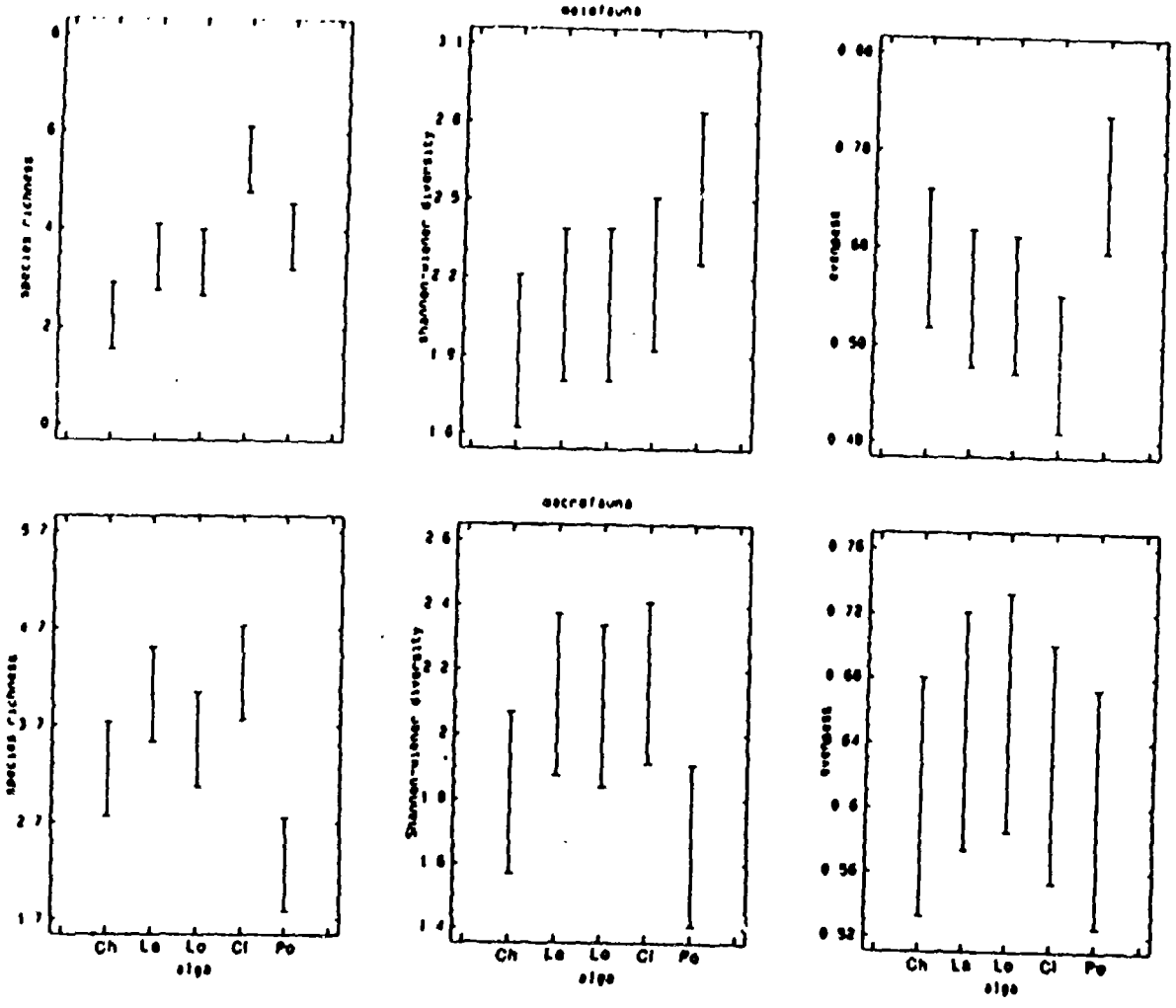


Fig. 14.12. Species richness (left), Shannon diversity (middle) and eveness (right) for meiofauna (top) and macrofauna (bottom), with 95% confidence intervals. Ch = Chondrus, La = Laurencia, Lo = Lomentaria, Cl = Cladophora, Po = Polysiphonia.

GRAPHICAL/DISTRIBUTIONAL PLOTS: k-dominance curves for meiofauna show only Polysiphonia with a distinctly lower curve than the other species. For macrofauna, curves not clearly distinguishable from each other.

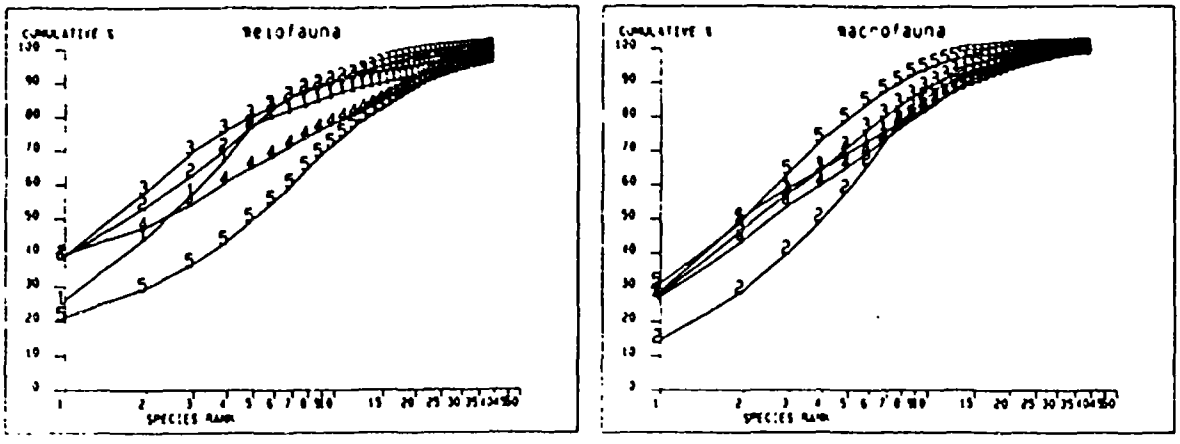


Fig. 14.13. k-dominance curves for meiofauna (left) and macrofauna (right). 1 = Chondrus, 2 = Laurencia, 3 = Lomentaria, 4 = Cladophora, 5 = Polysiphonia.

MULTIVARIATE ANALYSES: See Fig. 13.7. Two-way ANOSIM (weed species/sites) shows all weed species significantly different for both meiofauna and macrofauna. Note similarity of macrofauna and meiofauna configurations.

CONCLUSIONS: Multivariate methods more sensitive than univariate or graphical/distributional methods for discriminating between weed species. Univariate and graphical/distributional methods give different results for macrobenthos and meiobenthos, whereas for the multivariate methods the results are similar for both.

Example 6: Meiobenthos (nematodes and copepods) from the Tamar estuary, S.W. England (Austen & Warwick, 1989)

MAP OF SITES:

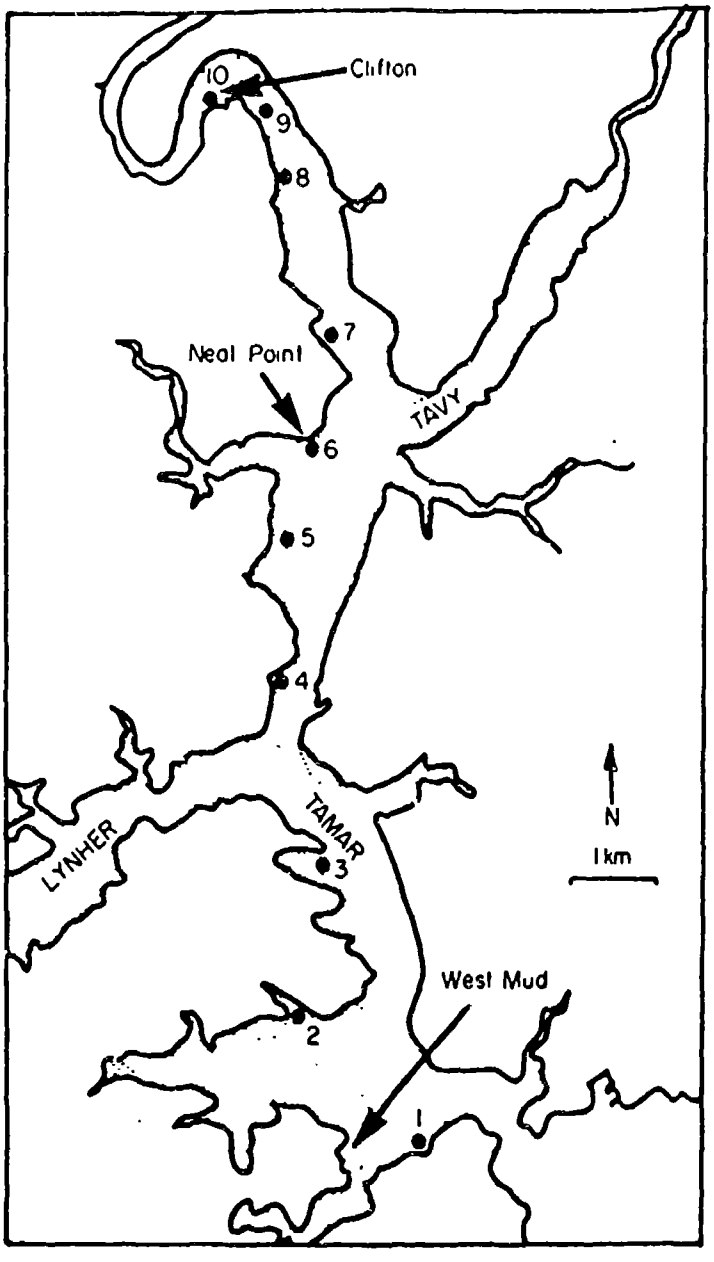


Fig. 14.14. Map of Tamar estuary showing locations of 10 intertidal mud-flat sites.

UNIVARIATE INDICES: Not determined.

GRAPHICAL/DISTRIBUTIONAL PLOTS: k-dominance curves for nematodes and copepods do not show similar sequence. For nematodes, sequence does not correspond to the salinity gradient, but for the copepods the agreement with salinity is closer.

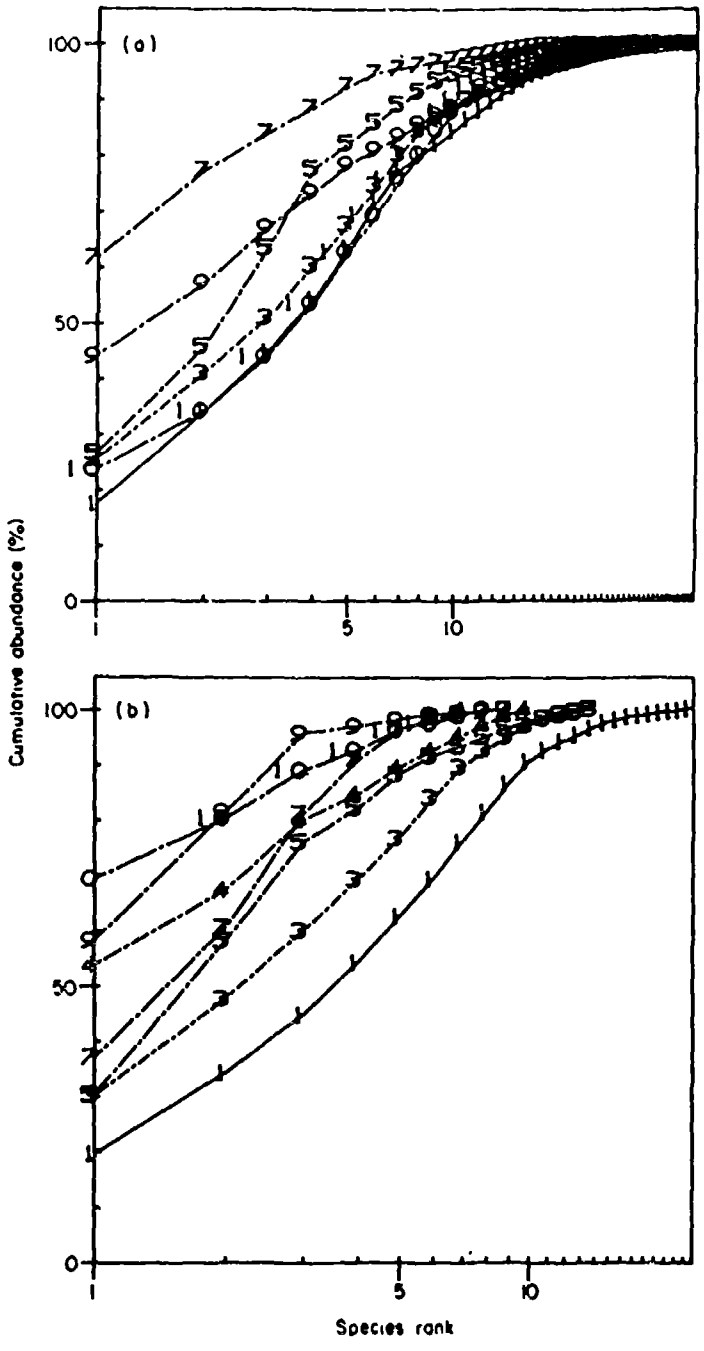


Fig. 14.15. k-dominance curves for amalgamated data from 6 replicate cores for nematodes (top) and copepods (bottom).

MULTIVARIATE ANALYSES: Sequence of sites ordered along the salinity gradient for both nematodes and copepods. ANOSIM shows copepod assemblages significantly different at all pairs of sites, nematodes at all pairs except 6/7 and 8/9.

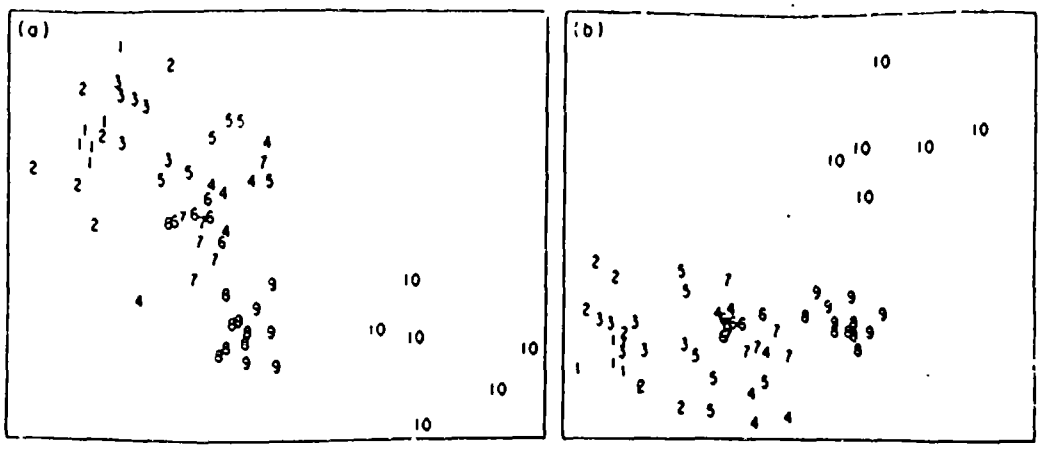


Fig. 14.16. MDS for nematodes (left) and copepods (right) for six replicate cores at each of 10 stations. Note that, allowing for the difference in orientation, the configurations are almost identical.

CONCLUSIONS: Multivariate techniques more sensitive in discriminating sites (many sites indistinguishable on basis of k-dominance curves). Multivariate methods give similar patterns for nematodes and copepods; graphical/distributional methods give different patterns for the two taxa. For nematodes, factors other than salinity are more important in determining diversity profiles, but for copepods salinity correlates well with diversity.

Example 7 Meiofauna from Tasmanian sandflat, influenced by burrowing and feeding of soldier crabs.

MAP OF SITES:
UNIVARIATE INDICES:
GRAPHICAL/DISTRIBUTIONAL PLOTS:

See Lecture 12

MULTIVARIATE ANALYSES:

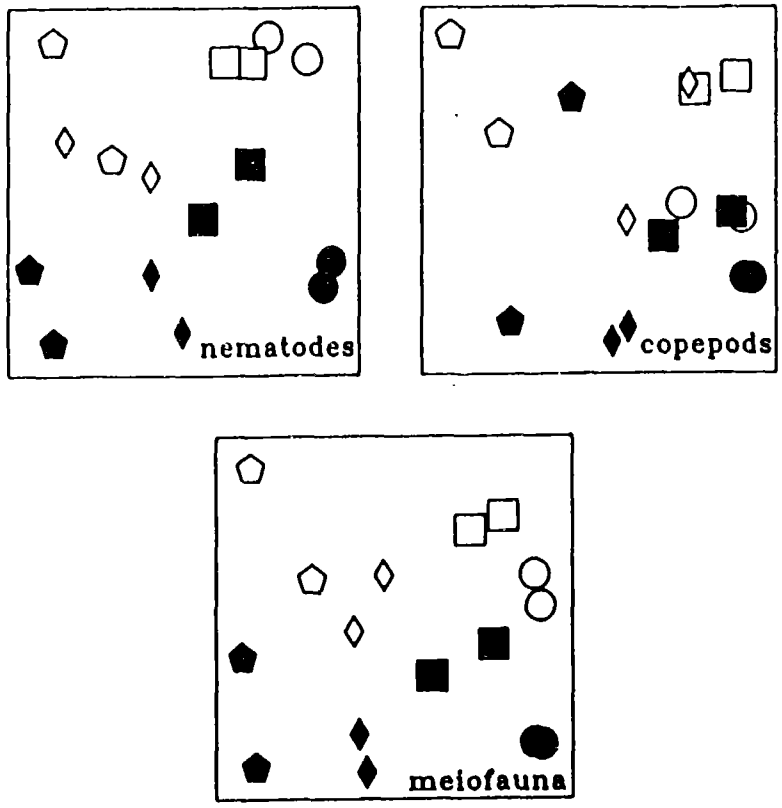


Fig. 14.17. MDS plots for nematode, copepod and 'meiofauna' (nematodes + copepods) abundance. Open symbols = disturbed samples, closed = undisturbed (different shapes denote the four blocks).

CONCLUSIONS: For nematodes, univariate, graphical/ distributional and multivariate methods all distinguish disturbed from undisturbed sites. For copepods only the multivariate methods do. Univariate and graphical/distributional methods indicate different reponses for nematodes and copepods; multivariate methods indicate a similar response.

GENERAL CONCLUSIONS

Three general conclusions emerge from these examples:

1. Similarity between sites based on their univariate or graphical/distributional properties is usually different from their clustering in multivariate analyses.

2. SPECIES DEPENDENT (multivariate) methods are much more sensitive than SPECIES INDEPENDENT (univariate and graphical/distributional) methods in discriminating between sites.

3. In examples where more than one component of the fauna has been studied, univariate and graphical/ distributional methods may give different results for different components, whereas multivariate methods tend to give the same results.

The sensitive multivariate methods are only capable of detecting differences in community composition between sites, although these differences can be correlated with measured levels of stressors such as pollutants. Only the species independent methods of data analysis can be used to determine deleterious (stress) responses. There is a need to develop techniques for determining stress which utilize the full multivariate information contained in a species/sites matrix.

RECOMMENDATIONS

At present, it is important to apply a wide variety of classes of data analysis, as each will give different information and this will aid interpretation. Sensitive multivariate methods will give an 'early warning' that community changes are occurring, but indications that these changes are deleterious are required by environmental managers, and the less sensitive species independent methods must be used.

FURTHER READING

For general texts on multivariate methods, the two books by Everitt (1978 and 1980) are useful introductions, and Chatfield and Collins (1980) can be recommended (though requires some knowledge of matrix algebra and statistical inference). A more detailed, but still approachable, exposition of MDS is the monograph by Kruskal and Wish (1978). (None of these texts is written from an ecological viewpoint.)

Papers which reflect the approach taken in these notes include Field et al. (1982), Warwick & Clarke (1991), papers from the GEEP Oslo Workshop Proceedings (e.g. Gray et al. 1988, Warwick et al. 1988, Clarke & Green 1988), and other methodological papers (e.g. Warwick 1986, Clarke 1990, Warwick & Clarke 1992 and Clarke & Ainsworth 1992).

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