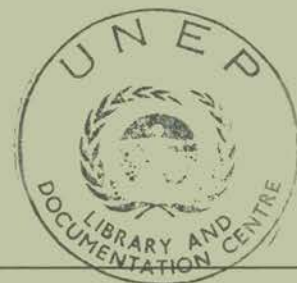




FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS



UNITED NATIONS
ENVIRONMENT PROGRAMME



COORDINATED MEDITERRANEAN POLLUTION MONITORING AND RESEARCH PROGRAMME (MED POL)

Report of the Mid-term Expert Consultation on the
Joint FAO (GFCM)/UNEP Coordinated Project on Pollution in the Mediterranean
(MED II, III, IV and V)
held in Dubrovnik, 2-13 May 1977



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS



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REPORT OF THE
MID-TERM EXPERT CONSULTATION
ON THE JOINT FAO (GFCM)/UNEP COORDINATED PROJECT ON POLLUTION
IN THE MEDITERRANEAN

Dubrovnik, 2-13 May 1977

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, May 1977

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PREPARATION OF THIS REPORT

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operating agency.

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REVIEW OF PILOT PROJECTS MED II
Baseline Studies and Monitoring of Metals, Particularly Mercury and Cadmium,
in Marine Organisms

AND MED III
Baseline Studies and Monitoring of DDT, PCBs
and other Chlorinated Hydrocarbons in Marine Organisms

Dubrovnik, 2-6 May 1977

INTRODUCTION

1. The Session was chaired by Prof. T. Balkas^{*}. Drs. A.P. Grimanis and H. Hornung agreed to act as Rapporteurs. The Agenda as reproduced in Annex I was adopted.
2. On behalf of the host country Prof. P. Strohal, Director of the Center for Marine Research, "Rudjer Bošković" Institute welcomed the participants (listed in Annex II).
3. As background to the Consultation, Dr. D. Charbonnier, Secretary of GFCM, made a brief historical review of the events which had led to the organization of the present meeting. He referred to the establishment by the Council in 1969 of a Working Party the activities of which culminated in the publication in 1972 of the review "The State of Marine Pollution in the Mediterranean and Legislative Controls". He underlined the recommendations formulated by the Council at its Eleventh and Twelfth Sessions in 1972 and 1974 respectively concerning the strengthening of international cooperation in the field of pollution in the Mediterranean and, more specifically, the implementation of a cooperative programme dealing with the effects of pollutants on marine communities and organisms as well as of a pilot project for the monitoring of certain contaminants in commercial species. The above recommendations contributed to the decision to organize in 1974 an International Workshop on Marine Pollution in the Mediterranean under the joint auspices of the Council, the United Nations Environment Programme, the International Commission for the Scientific Exploration of the Mediterranean and the Intergovernmental Oceanographic Commission. This meeting identified a series of seven pilot projects and GFCM agreed to coordinate the implementation of four of them. The programme as drawn up during the meeting has been endorsed at the beginning of 1975 as part of an Action Plan by the UNEP Intergovernmental Meeting on the Protection of the Mediterranean and a FAO(GFCM)/UNEP Expert Consultation was convened that same year to prepare, for each of the four pilot projects, an operational document which is now an integral part of the agreements concluded between those research centers which participate in these projects and FAO(GFCM).
4. Dr. S. Kečkeš, Programme Coordinator of the Mediterranean Pollution Monitoring and Research Programme, greeted the participants on behalf of UNEP and expressed UNEP's hope that the successful collaboration established between research centres participating in MED II and MED III, FAO(GFCM), IAEA and UNEP will bring its first visible fruits at this meeting.
5. He reviewed the components of the Action Plan adopted by the Governments of the Mediterranean countries at the Intergovernmental Meeting on the

^{*}Prof. P. Strohal served as acting Chairman on 2 May.

Protection of the Mediterranean Sea (Barcelona, January/February 1975) and the progress achieved in its implementation (document FIR:PM/77/3). He stressed that the assessment of the present level of pollutants in the Mediterranean and of their impact on marine environment and human health, which will be carried out through the pilot projects of the Coordinated Mediterranean Pollution Monitoring and Research Programme (referred to as MED POL), is an integral part of this Action Plan. It serves as the scientific basis for technical, administrative and legal actions which might be undertaken by the Governments of the region for the improvement of the quality of the Mediterranean environment.

6. Dr. Kečkeš indicated the following tasks for the meeting:

- (i) to review the results of the first phase of MED II and MED III;
- (ii) to prepare a first evaluation of pollution in the Mediterranean by selected metals and chlorinated hydrocarbons;
- (iii) to clarify methodological questions and to improve the implementation of the second phase of MED II and MED III, using the Operational Document (document FIR:PM/77/4 as the framework for these pilot projects.

The results presented at this meeting will be integrated with those obtained from similar meetings on the other pilot projects of MED POL and submitted to an Intergovernmental Meeting of the Mediterranean Countries (Monaco, 28 November - 6 December 1977) as the report on the state of pollution in the Mediterranean region.

7. Mr. A. Wenblad of the FAO(GFCM) Secretariat gave a general review of the progress of the pilot projects MED II and MED III outlining as a background the steps that had been taken for the execution of the projects following the development of the Operational Document. Following UNEP's request of 1 September 1975 to the Mediterranean Governments 38 national research centres from 14 countries had been designated as participants in MED II and 28 centres from 13 countries as participants in MED III. Fifty two agreements, giving details about the scientific work to be carried out as part of MED II and MED III and the assistance to be received had subsequently been prepared between FAO(GFCM) and the research centres.

8. Most of the main instruments (atomic absorption spectrophotometers and gas chromatographs) have now been delivered and several of them are operational. A common maintenance service had been arranged by UNEP through the IAEA Laboratory in Monaco making a service engineer available for this purpose. The participants were asked to contact UNEP or FAO(GFCM) when there are service needs which cannot be solved locally; keeping in mind that the servicing of instruments provided through the pilot projects has priority.

9. An important activity of the pilot projects was the training component. Of the 40 training opportunities of various duration several had been initiated or are already completed. All the training was performed in the Mediterranean area and, with a few exceptions, in research centres participating in the pilot projects. A manual on sampling and analyses of biological material, a selected bibliography on studies and research relevant to pollution and two newsletters have been prepared for the benefit of the participants in the pilot projects.

10. The task of the Regional Activity Centres for MED II and MED III (Center for Marine Research, "Rudjer Bošković" Institute, Rovinj/Zagreb, and Marine Science Department, Middle East Technical University, Ankara)

has been agreed upon in discussions between these centres and FAO(GFCM) and UNEP. FAO(GFCM) and UNEP expect that these Regional Activity Centres will provide a valuable assistance in the execution of the pilot projects.

11. It was requested that the research centres participating in the pilot projects follow the mandatory part of the programme as agreed in the operational document (document FIR:PM/77/4). Any participant who is willing to do more than the mandatory part of the programme is encouraged to:

- (i) analyze additional marine organisms and pollutants;
- (ii) investigate pollutant levels in sea water and sediments;
- (iii) research biogeochemical processes of pollutants (including anthropogenic relationships).

Such research will be helpful for making specific recommendations to the Governments regarding future projects.

12. In view of the late start of work it was strongly recommended that the project be extended to the end of 1978.

13. Principal investigators were reminded that agreements provide for submission to FAO(GFCM) of quarterly monitoring results and semi-annual progress reports. Participant having difficulties interpreting analytical results may obtain assistance from FAO(GFCM), the relevant Regional Activity Centre or an expert of another research centre participating in the research project.

14. It was suggested that FAO(GFCM) should assure that all research centres are informed of pilot project progress on a semi-annual basis.

STATISTICAL CONSIDERATIONS IN DATA GENERATION AND PROCESSING

15. Statistical considerations in data generation and processing were presented by Prof. F. Möller (document FIR:PM/77/6), to demonstrate the usefulness and efficiency of utilizing statistical techniques to reduce the number of analyses required and to obtain the greatest possible evaluatory power from those data produced. It is clear that a functional relationship exists between the accuracy of the estimate of any population parameter and the number of observations with the sampling error decreasing as the number of observations increases. The central limit theorem provides assurance that the estimates are the best. If an infinite number of observations are made, the error of the estimation must be zero; that does not mean that all sample sizes must be very large. On the contrary, statistics has developed techniques with and about small sample sizes. It should also be clear that, if observed data are not precise and accurate no statistical method can correct them, with or without the most sophisticated computer programmes. As to the establishment of an optimum sample size (number of observations) this can only be determined following pilot sampling and determination of the appropriate variances - importance of factors in terms of their variability of error.

16. Discussion of this presentation was extensive especially on the topics of quality control (regarding number of pretreatments and readings) and the use of composite samples. It led to the formation of a working

group. This group expressed the following:

- (i) For the time being the use of homogenates (composite samples) raises problems from analytical and statistical points of view. Namely, homogenization may introduce considerable contamination of metals analyzed, if not properly performed and it is difficult to obtain complete homogenization and a representative sample. For trace organics, contamination plays a minor rôle and the obtainable homogeneity seems to have the dominant influence. Therefore, to avoid absolutely unknown errors, at the present stage of the project the analysis of single specimens would be desirable. Besides the routine analyses, some laboratories with experience in this field should conduct limited pilot studies on the problems of homogenization.
- (ii) Being aware of the necessity to have accurate results in the final assessment of the contamination of the Mediterranean (where the differences between either individual or pooled samples from various areas is of prime importance) minor systematic errors can be tolerated, provided that they act, for a defined population and/or a set of measurements, in the same direction. When reference materials resembling as closely as possible in their composition the tissues analysed in this project, become available (e.g. oyster homogenate when certified) systematic errors could be corrected by running representative samples in the same way and in the same conditions.

17. In response to the recommendation by the working group that individual specimens be analysed wherever possible further discussion was stimulated. The resulting agreement was that composite samples will continue to be used, although at least once a year individuals will be analysed to determine the variability between organisms. The merit of selecting a common sampling period for analysing individual variability was accepted (July sampling period).

REVIEW OF PILOT PROJECT MED II

18. The summary of the results reported by the principal investigators (document FIR:PM/77/7) were reviewed and discussed. Several investigators participating in this project presented additional results; summaries of their presentation are included in Annex IV. Conclusions about the pollution level of the Mediterranean cannot be drawn at this stage of the project. However, mercury data presented at this Consultation have extended previous knowledge, especially for crustaceans and fishes. Results of mussel monitoring efforts confirmed previously reported values. Relatively high mercury levels in predatory cephalopods (*Sepia* and *Octopus*) are thought to justify encouraging research centres to include them, where available, in their monitoring efforts. Results indicate that the selection of a widely available species of crustacean for monitoring by all research centres has not been successful. Mercury levels reported for *Mullus barbatus* appear to reflect local differences in exposure; the fact that there were no published values for this species increases the importance of MED II results. MED II analyses of *Thunnus thynnus* confirm earlier reports of high values of mercury for this species. Other fishes for which several values were reported were *Merluccius merluccius*, *Sardina pilchardus* and *Engraulis encrasicolus*.

19. The consistent monitoring of designated species following installation of instruments at all research centres and completion of training programmes should expand the data base rapidly for mercury and cadmium as well as additional elements. The need does exist, however, to extend the monitoring effort to include organisms along the coasts of areas not now covered.

20. The need was also expressed for data on sediment, water and air-water interface to contribute to the explanation of the transport and accumulation of the contaminants in the ecosystems. It was agreed that this work was an important voluntary component of the pilot project and that FAO(GFCM) would coordinate the activities of the involved research centres. Dr. A. Grimanis presented to the Consultation some results of his group's studies on heavy metals in sediment. Dr. M. Bernhard presented a systematic survey on the levels of potential pollutants in the Mediterranean Sea (document FIR:PM/77/9).

21. Dr. C. Osterberg, Director of the IAEA International Laboratory for Marine Radioactivity reviewed the results obtained by several participating research centres in the intercalibration exercise for trace elements using oyster homogenate [MA-M-1] (document FIR:PM/77/10). The exercise was successful and will be furthered by use of the fish homogenate which will soon be ready.

22. Participants in the MED II pilot project were reminded that they are obliged to participate and complete all intercalibration exercises organized by the Monaco International Laboratory. Participants were very grateful to this laboratory for providing this service because it gives them confidence in methods they use in the analysis of trace elements in marine organisms. The Monaco laboratory is also developing an analytical technique for lead which will be available to participants.

23. Participants were encouraged when they submit reports to FAO(GFCM) or publish papers related to trace elements in marine organisms to refer to the Monaco Intercalibration Exercise. If centres are not satisfied with their results in the exercise both FAO(GFCM) and the Monaco laboratory will provide assistance upon request.

24. Participants were urged to comment on the Manual of Methods in Aquatic Environment Research, Part 3, Sampling and Analyses of Biological Material, by M. Bernhard, FAO Fish.Tech.Pap., (158). Comparison of classical wet digestion procedures and digestion with teflon bomb system was made for nanogram levels of some volatile trace elements; the digestion with the teflon bomb system is preferable. For higher levels of trace elements both methods are satisfactory. The potential of electroanalytical methods for the analysis of sea water was pointed out. Such methods are available for the analysis of limited numbers of trace elements (Cd, Cu, Pb, Zn and Hg) in sea water. For the determination of trace elements in marine organisms the recommended reference method specified in the operational document is atomic absorption spectrophotometry. A working group on analytical methodology was established; it will prepare and submit to FAO (GFCM) detailed comments on analytical procedures, to be distributed to participants.

25. Sufficient data must be reported regarding origin, size, (length and weight), general appearance (i.e. health), date and area collected, sexual maturity and when possible age of organisms analysed.

26. FAO(GFCM) will continue to supply assistance with equipment, training, experts, intercalibration, maintenance of apparatus and analytical services.

REVIEW OF PILOT PROJECT MED III

27. Results on baseline studies and monitoring of DDT, PCBs and other chlorinated hydrocarbons in marine organisms included in document FIR:PM/77/7 were presented by Dr. T. Söylemez of the Regional Activity Centre, and discussed. Data were scarce as most laboratories in this project have only recently gained analytical capability. Only three areas of the Mediterranean are covered by results now available. It was suggested that the number of sampling stations should be increased, as well as the number of species investigated. Results are to be submitted after each sampling period.

28. Additional data, not included in document FIR:PM/77/7, were discussed. Summaries of the presentations are included in Annex V. The preliminary results indicate that seasonal variations may occur in some of the monitored organisms. It was suggested that the number of sampling sites be increased and where necessary (because of absence of organisms) changed. It can be seen from available data that the open sea has lower levels than the coastal area.

29. The need was expressed to develop information on the transport and accumulation of chlorinated hydrocarbons in sediments, water, and especially at the air-water interface. Involvement of research centres in this voluntary component of the programme will be coordinated by FAO(GFCM).

30. Because of maintenance problems (i.e. repair of the electron-capture detector) many laboratories did not present any data, although the collection of samples was carried out as planned and samples were stored. FAO(GFCM) has agreed to arrange for analysis of samples for those laboratories who need and request this service.

31. Two methods of extraction of the homogenate were discussed relative to the Manual of Methods in Aquatic Environment Research, Part 3, pp. 89-90, the blender and the soxhlet extraction. A working group was appointed to determine the best extraction method to apply, type of solvent to be used, use of sulfuric acid, and suggestions for evaluating the chromatograms (i.e. isomers). Specific proposals for amendments to the Manual were left with the Secretariat.

32. Sufficient data must be provided regarding size (length and weight), general appearance (i.e. health), sexual maturity, and when possible age of organisms analysed. These data will be useful for the intercomparison of the results.

33. Dr. C. Osterberg reported that only one laboratory submitted the results for the intercalibration exercise, so that no compilation was possible. Other centres were urged to complete this exercise as soon as possible.

34. For confirmatory analyses and investigation of unusual results it was suggested that GC/MS be utilized. Research centres are encouraged to develop this analytical technique. There are GC/MS laboratories in Montpellier, France; Athens, Greece; Padua, Italy and Zagreb, Yugoslavia. An agreement could be explored with these centres to enable the use of their GC/MS units, or another laboratory could be established which would

be devoted to efforts supportive of the pilot projects.

35. It was suggested that FAO(GFCM) should supply standards (preferably in solid state) or inform the participating laboratories of the various suppliers and other relevant information (i.e. time required for delivery). A number of available suppliers were discussed.

REPORTING SYSTEM

36. Evaluation and processing of collected data was reviewed. A special format for data collection and reporting was suggested as a uniform model to avoid loss of data and for comparison purposes. The importance of presenting analytical results in a common manner was discussed and participants were reminded that the operational document calls for reporting on both a wet and dry weight basis ($\mu\text{g}/\text{kg}$) and for chlorinated hydrocarbons the extractable organic material will be determined. Further comments of the participants on the suggested format will be accepted by FAO(GFCM), evaluated, and a revised version submitted to all research centres.

37. Original raw data collected by the participants will be considered confidential. Results reported by research centres on a quarterly basis to FAO(GFCM) will be evaluated with the assistance of the appropriate Regional Activity Centre, but will not be otherwise distributed or published without approval of the relevant research centre. FAO(GFCM) will after evaluation of the results provide an appraisal of the pollution situation in the Mediterranean. FAO(GFCM) will not distribute or publish these confidential data.

38. Participants are also encouraged to submit their results to scientific journals acknowledging their involvement in the project and requesting a waiver of copyright by the publisher for UN use of the data (similar to United Kingdom and USA Government prerogatives). The suggested wording for acknowledgement and copyright request is found in document FIR:PM/77/5.

39. The GFCM Reports will continue to contribute to communication between project research centres. In addition, a UNEP newsletter dealing with recent achievements within the Mediterranean Action Plan is being initiated. In the future the GFCM Report will contain an annex citing recent publications of project investigators. UNEP plans to publish annually collected reprints dealing with pollution.

40. A selected bibliography of research relevant to pollution in the Mediterranean (FAO Fish.Tech.Pap., (165) was published and presented. The regional computed bibliography will be updated and used with the assistance of principal investigators and Regional Activity Centres. Aquatic Science and Fisheries Abstracts (ASFA) can be used as an additional source of information. It was noted that ASFA is being computerized and will be accessible through an information retrieval system. FAO(GFCM) is willing to provide photocopies or microfilms of papers not available to investigators.

FUTURE ORIENTATION AND RECOMMENDATIONS

41. In view of the late start of sampling and analytical work and in order to ensure an adequate geographical coverage, the Consultation strongly recommended to extend the duration of pilot projects MED II and MED III to the end of 1978.

42. In order to expand the area covered so far, a 1978 joint scientific cruise covering international waters of the Mediterranean is under investigation and should be arranged. A programme should be outlined by the relevant Specialized Agencies and an organizing committee composed by competent and experienced scientists. Technical aspects will be investigated if and when a sufficient number of participants is interested. It was recommended that the primary goal be training with emphasis on sampling techniques, field data collection, handling of samples, avoidance of contamination, etc. The sampling should include water, sediments and biota. It was suggested that the most suitable ship for the outlined purposes would be a fishery research vessel.

43. It was recommended to request the biologists attending the Expert Consultation on the review of projects MED IV and MED V to establish a list of alternative species for monitoring where the species listed in the operational document are not available.

44. Every participating laboratory is encouraged to determine more components (substances and species) than in the obligatory programme (document FIR:PM/77/4). All participants are reminded that the components in the obligatory programme should not be neglected.

45. The proposal for the "Mussel Watch" experiment (document FIR:PM/77/11) was adopted and the participants in MED II and MED III were invited to participate in it on a voluntary basis.

46. In relation to the Unesco paper "The Use of Modelling of Marine Systems in the Framework of UNEP Monitoring and Research Programme", by L. Jeftić and T. Legović, made available at the Consultation, the modelling of biogeochemical cycles of pollutants, in particular mercury, was accepted as optional activity in the framework of MED II and MED III.

47. Other optional programmes suggested were air sampling of chlorinated hydrocarbons and fluxes of metals between ecosystem compartments.

ADOPTION OF THE REPORT AND CLOSING OF THE SESSION

48. The Report was adopted on 6 May 1977. The Chairman asked Prof. P. Strohal to receive on behalf of the Host Country the appreciation of the Consultation for the excellent technical arrangements. The Consultation was closed on 6 May 1977.

REVIEW OF PILOT PROJECTS MED IV
Research on the Effects of Pollutants on Marine Organisms
and their Populations

AND MED V
Research on the Effects of Pollutants on Marine Communities and Ecosystems

Dubrovnik, 9-13 May 1977

INTRODUCTION

49. The Session was chaired by Prof. J. Štirn. Drs. G. Bellan and R. Semroud agreed to act as Rapporteurs. The Agenda as reproduced in Annex I was adopted.

50. On behalf of the host country Prof. P. Strohal, Director of the Center for Marine Research, "Rudjer Bošković" Institute welcomed the participants (listed in Annex II).

51. As background to the Consultation, Dr. D. Charbonnier, Secretary of GFCM, made a brief historical review of the events which had led to the organization of the present meeting. He referred to the established by the Council in 1969 of a Working Party the activities of which culminated in the publication in 1972 of the review "The State of Marine Pollution in the Mediterranean and Legislative Controls". He underlined the recommendations formulated by the Council at its Eleventh and Twelfth Sessions in 1972 and 1974 respectively concerning the strengthening of international cooperation in the field of pollution in the Mediterranean and, more specifically, the implementation of a cooperative programme dealing with the effects of pollutants on marine communities and organisms as well as of a pilot project for the monitoring of certain contaminants in commercial species. The above recommendations contributed to the decision to organize in 1974 an International Workshop on Marine Pollution in the Mediterranean under the joint auspices of the Council, the United Nations Environment Programme, the International Commission for the Scientific Exploration of the Mediterranean and the Intergovernmental Oceanographic Commission. This meeting identified a series of seven pilot projects and GFCM agreed to coordinate the implementation of four of them. The programme as drawn up during the meeting has been endorsed at the beginning of 1975 as part of an Action Plan by the UNEP Intergovernmental Meeting on the Protection of the Mediterranean and a FAO(GFCM)/UNEP Expert Consultation was convened that same year to prepare, for each of the four pilot projects, an operational document which is now an integral part of the agreements concluded between those research centres which participate in these projects and FAO(GFCM).

52. Dr. S. Kečkeš, Programme Coordinator of the Mediterranean Pollution Monitoring and Research Programme, greeted the participants on behalf of UNEP and expressed UNEP's hope that the successful collaboration established between research centres participating in MED IV and MED V, FAO(GFCM) and UNEP will bring its first visible fruits at this meeting.

53. He reviewed the components of the Action Plan adopted by the Governments of the Mediterranean countries at the Intergovernmental Meeting on the Protection of the Mediterranean Sea (Barcelona, January/February 1975) and the progress achieved in its implementation (document FIR:PM/77/3). He stressed that the assessment of the present level of pollutants in the Mediterranean and of their impact on marine environment and human health, which will be carried out through the pilot projects of the Coordinated

Mediterranean Pollution Monitoring and Research Programme (referred to as MED POL), is an integral part of this Action Plan. It serves as the scientific basis for technical, administrative and legal actions which might be undertaken by the Governments of the region for the improvement of the quality of the Mediterranean environment.

54. Dr. S. Kečkeš indicated the following tasks for the meeting:

- (i) to review the results of the first phase of MED IV and MED V;
- (ii) to clarify methodological questions (such as guidelines for toxicity tests, methods for selection of species or processes indicating marine pollution, etc.) and thus to improve the implementation of the second phase of MED IV and MED V, using the Operational Document (document FIR:PM/77/4) as the framework for these projects;
- (iii) to provide input into the monitoring pilot projects of MED POL (MED II and MED III in particular), into the planned predictive Mediterranean model of the biogeocycle of selected pollutants, and into other activities carried out as part of the Action Plan (such as the development of ecological rationale for the Protocol for the Protection of the Mediterranean Sea Against Pollution from Land Based Sources, of guidelines for the selection of specially protected areas etc.).

The results presented at this meeting will be integrated with those obtained from similar meetings on the other pilot projects of MED POL and submitted to an Intergovernmental Meeting of the Mediterranean Countries (Monaco, 28 November - 6 December 1977) as the report on the state of pollution in the Mediterranean region.

55. Mr. A. Wenblad of the FAO(GFCM) Secretariat gave a general review of the progress of the pilot projects MED IV and MED V outlining as a background the steps that had been taken for the execution of the projects following the development of the Operational Document. Following UNEP's request of 1 September 1975 to the Mediterranean Governments 23 national research centres from 10 countries had been designated as participants in MED IV and 20 centres from 10 countries as participants in MED V. Twenty eight agreements, giving details about the scientific work to be carried out as part of MED IV and MED V and the assistance to be received have subsequently been prepared between FAO(GFCM) and the research centres.

56. Main instruments (atomic absorption spectrophotometers and gas chromatographs) provided for pilot projects are expected to also allow analyses on levels of contaminants studied in the research projects MED IV and MED V. The equipment ordered and in many cases already delivered for the pilot projects MED IV and MED V cover a wide range, from sophisticated laboratory instrumentation to field equipment like dredges and rubber boats.

57. An important activity of the pilot projects is the training component. Of the 18 training opportunities of various duration several have been initiated or are already completed. All the training was performed in the Mediterranean area and, with a few exceptions, in research centres participating in the pilot projects. A manual on sampling and analyses of biological material for metals and chlorinated hydrocarbons, a selected bibliography on studies and research relevant to pollution and two newsletters have been prepared for the benefit of the participants in the pilot projects.

58. The agreement between FAO(GFCM), UNEP and the Regional Activity Centre for MED V, Centre de recherches océanographiques et des peches, Alger has recently been prepared while the agreement with the Regional Activity Centre for MED IV, Station marine d'Endoume et centre d'océanographie, Marseille is under preparation. It is expected that FAO(GFCM) and UNEP will receive valuable assistance from these centres in the execution of the pilot projects.

59. Document FIR:PM/77/12 dealing with the designation of marine parks - natural reserves was elaborated on and a working group was established to deal with the question.

60. On delivery of the report of that group the significance of marine parks and reserves as reference sources for scientific research, especially monitoring and research programmes was stressed. It was noted that, for comparison purposes, such parks and reserves considered as reference zones should therefore present at least two essential characters, i.e. ecological and hydrological. It should then be possible to undertake in these so called reference zones research and monitoring activities dealing with: communities (benthic-planktonic), hydrology, biogeochemistry, other species. It was recommended to increase the number of so called "clean" reference zones and to investigate them in relation with biocoenose studies being carried out in polluted zones. Additional information on Mediterranean protected areas was provided by participants for transmission to IUCN.

61. It was suggested that participants in MED V provide assistance in developing guidelines and principles for the selection and management of protected marine areas and in the selection of these areas by their national authorities.

62. Exploration of a MED POL cruise for 1978 (spring-autumn) was reviewed with the question of beneficial use assigned to a working group, as were the questions of contributing to the development of a biogeochemical model and the construction of a list of alternative species for use in MED II and MED III when and/or where obligatory species are unavailable (see paragraphs 105-108 and 74).

63. The "black" and "grey" lists of the "Protocol for the Prevention of Pollution of the Mediterranean Sea by Dumping from Ships and Aircrafts" and of the "Draft Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources" were discussed from the position of uniform principles which might be utilized in establishing discharge standards (i.e., regional waste receiving capacity, harm to man and environmental degradation). This too was assigned to a working group for further development (see paragraphs 109-111).

64. There was a consensus that MED POL should be extended for a minimum of one year (1978). The completion of essential training, conclusion of data collection, and extension of required funding were all topics of concern.

65. Wide geographic gaps in MED IV and MED V caused concern. The hope was expressed that additional countries and research centres may become involved.

66. Dr. H. Naeve introduced part I of document FIR:PM/77/13, the review of pilot projects MED II and MED III, which was briefly discussed.

REVIEW OF PILOT PROJECT MED IV

67. Results obtained during the first phase of the pilot project MED IV, "Research on Effects of Pollutants on Marine Organisms and Their Populations" were presented by Dr. G. Bellan of the Regional Activity Centre and discussed. Presentation was done following the form of results presented in the progress report prepared by FAO(GFCM) (document FIR:PM/77/8): Toxicity - Pollutant Dynamics - Physiological and Behavioural Effects - Morphology and Histopathology - Development, Reproduction and Population Genetics.

68. Additional data, not included in the document were presented and critical points emphasized by principal investigators. Summaries are included in Annex VI. Each principal investigator emphasized the most important points of the research carried on by their laboratory.

69. Discussion was developed on several points raised during the presentation of the results, focusing particularly on the applicability of the results obtained for an early warning monitoring system, the possible use of biological systems to detect levels of pollutants chemically undetectable and to understand the mode of action of the contaminants.

70. Review of research variables within the frame of the project was presented by Dr. D. Johnson. The review took into account species used, water quality properties, care and maintenance of test animals, level of contaminants used, etc.

71. The subsequent discussion focused on the problems encountered using artificial sea water in comparison to natural water; the advantages are ability to control chemical composition and to have uniformity of water quality between projects and centres, while natural water is much more stable chemically and available in relatively unlimited quantity.

72. The maintenance of laboratory test animals and the special care that marine animals need was discussed with emphasis on the importance of initial condition (sampling techniques and transport), water quality, handling and maintenance procedures (to reduce as much as possible the introduction of stress). Stress may reduce resistance to potential pathogens and the result is an insurgence of disease.

73. Importance, significance and experimental designs using early life stages of marine animals were discussed and practical advantages, such as number of experiments, small volume of experimental water for the test and high sensitivity of the stages were underlined.

74. A discussion was also developed on the species to be used in toxicological tests, criteria to be utilized for this choice were discussed as well as practical implications and will be presented in recommendations. A list of alternative monitoring species was also developed in response to the recommendation of the Consultation on MED II, MED III (see paragraph 43) for use when obligatory species are unavailable (Annex VIII).

75. Points regarding chemical characteristics of the test water and water solubility of the contaminants were raised, particularly on chemical equilibria, since the chemical form may be critical to determining the biological effects. A recommendation on the importance of the knowledge of this point has been prepared.

76. A review of research approaches, such as bioassay methods, morphology and histopathology, development etc. were presented by Dr. D. Calamari analysing values and limitations of each approach. Main points discussed were:

- (i) The sensitivity of life cycle tests, and the importance of the studies on pollution dynamics as background to understand the results produced in the framework of the projects MED II and MED III (i.e. the rate of uptake (assimilation) and excretion of contaminants by the animals monitored).
- (ii) Need for uniformity of methodologies and common operational principles were also stressed, as results of this discussion a recommendation has been prepared for the production of a technical manual containing instructions for various types of tests.
- (iii) The need for an intercalibration exercise for biological tests was also raised in order to better compare the results and check the techniques.
- (iv) Development of assistance programmes was reviewed by Mr. A. Wenblad discussing assistance given and inviting participants to identify further needs. Points raised were regarding practical difficulties with instruments and training. The need of experts to assist research projects in unexperienced centres was also mentioned.

77. In view of the future phase of the project MED IV a critical evaluation of the results was accomplished mainly in relation to the objectives for which such information would be used. Schematically the goals of project MED IV are three:

- (i) Evaluation of the consequence of the pollution load and prediction for the future.
- (ii) Development of new methodologies.
- (iii) Support in understanding the results of MED II, III and V projects.

78. The contribution produced until now at the participating centers was considered useful and of good scientific level but not yet adequate to meet the goals listed above. Too many species have been considered in this first phase of the project; this has reduced the potential importance of the results produced until now. A need for better coordination and restriction of the field of the research approaches was identified, concentrating particularly on efforts with potential to support and explain the results obtained in MED II, III and V.

79. Two main reasons were identified as contributing to the described situation: the scarcity of laboratories with experience available to participate and the resultant high percentage of participating centres in MED IV now establishing laboratories and obtaining training, and second the enormous task and the wide range of fields that have to be covered.

REVIEW OF PILOT PROJECT MED V

80. A summary of results concerning research on the effects of pollution on marine communities and ecosystems was presented by Dr. R. Semroud,

Regional Activity Center; studies on the structure of benthic communities, as well as on qualitative and quantitative variations in planktonic communities, demonstrated the modification of these communities by various pollutants. Experimental studies have also been undertaken to investigate the effects of pollution on controlled ecosystems.

81. Additional data not provided in documents FIR:PM/77/8 were given by principal investigators present and discussed. They are summarized in Annex VII. The number of these data is rather low due to the delayed start of research and to the time-consuming nature of the ecological approach.

82. It was noted that as part of MED V work is being carried out or is planned to be started soon in the following geographical areas: North-Western Basin (France), Adriatic Sea (Yugoslavia), Aegean Sea (Greece, Turkey), Levantine Basin (Cyprus, Egypt), North African Waters (Algeria). The Consultation noted that, nevertheless, Mediterranean coverage is still inadequate, since large zones are not included in the project.

83. Ecosystems under influence of several specific types of pollution are being investigated, such as oil refineries, paper mills, match-fabrication and mining effluents, yet in the most cases research is dealing with the consequences of domestic sewage, food-processing and similar biodegradable effluents. In particular cases (Saronikos Bay, North Adriatic and Marseilles) consequences of complex urban-industrial mixed effluents are also being studied.

84. The majority of projects are investigating ecosystem-community modifications due to the local pollution sources in comparison with conditions in closely located but clean areas. For the above mentioned cases of large pollution nuclei, complex investigations are on the way. Some projects focused their attention on experimental approaches of the assessment of pollution consequences, such as fouling tests and experiments in artificially polluted semi-natural coastal environments.

85. Techniques used for the study of these communities are extremely varied. Besides standard measurements of the environment, pelagic and benthic sampling have been carried out by different methods. The comparability of results is therefore dubious.

86. The consultation has been able to evaluate the above observations and wishes to emphasize a number of significant points:

- (i) Research on ecosystems and more particularly on communities in the framework of pollution evaluation have been not only pursued in areas previously studied, but have also been initiated in several sectors previously neglected.
- (ii) Results obtained by the various projects provide a new insight into Mediterranean ecosystems.
- (iii) This research has been initiated in some areas (North Africa and Near East coasts) which are distant from important pollution sources and are only affected by local pollutants.
- (iv) A particularly welcome change is the progressive orientation toward the establishment of controlled ecosystems.

87. However, the Consultation does recognize some deficiencies:

- (i) First the geographical incompleteness of studies.
- (ii) The difficulties encountered in the determination of relationships between the observed effects and their causes, as well as establishing relationships between field and laboratory results.
- (iii) The lack of thorough studies in general ecology. This appears to be the cause of difficulties encountered in the approach to MED V theme.
- (iv) Technical problems related to sampling, analysis and presentation of results.
- (v) All of these problems slow down the study of ecosystems.

88. An instructive discussion on the methodology to be used in the study of colonization and development of plate fouling communities occurred. It was generally agreed that this technique should be encouraged, but some reservations were expressed about the interpretation of results, particularly as regards the evaluation of pollution levels.

89. After a brief review of the Mediterranean situation, Prof. J. Štirn proposed to discuss the methodology concerned in determining of the structure of communities (e.g. problems of sampling, biomass, fouling, etc.).

90. A discussion followed concerning biostimulation tests on algal cultures and the relationship between nutrients and development of these algae: eutrophication. Although some laboratories have already attempted to study these complex problems, concern was expressed about the lack of interest for these questions in general.

91. As regards this approach to research, Dr. G. Bellan insisted in the interpretation of their results (dynamics, productivity and biomass must be considered). He mentioned theoretical and practical problems encountered with these research approaches and underlined the limitations of the methodologies used or advocated.

92. In order to better understand natural phenomena, an experimental approach to research problems should be able to draw on both pilot projects MED IV and MED V since field observations and laboratory tests not supported by field experiments have limited value.

93. Although the utilization of pollution indicators both in benthology and planktonology calls for the participation of taxonomists, it would appear easy to obtain a general picture of the pollution from their use. Diversity indices are of definite interest.

94. In view of the complexity of the study on marine communities and ecosystems, participants in general expressed the hope that the coordinated programme could be extended throughout 1978. It was further suggested to prepare a Manual on guidelines for community and ecosystem studies, preferably through assistance of the Regional Activity Centre for MED V.

95. Mr. A. Wenblad briefly reviewed the assistance given by FAO(GFCM)/UNEP to the programme of pilot project MED V.

REPORTING SYSTEM

96. Principal investigators were reminded that agreements provide for submission to FAO(GFCM) of semi-annual progress reports and annual research reports. Participant having difficulties interpreting analytical results may obtain assistance from FAO(GFCM), the relevant Regional Activity Centre or an expert of another research centre participating in the research project.

97. Problems raised by storage, evaluation and processing of collected data were reviewed. A uniform model for collecting and reporting data will be suggested to avoid loss of data and facilitate comparisons. The importance of presenting results of observations, analyses or determinations in a common manner was stressed. It was suggested that semi-annual reports should be highly technical and deal primarily with conditions and methods of experiments, analyses, or field studies, whereas the annual report should be in scientific manuscript format.

98. In order to elaborate complete forms concerning the experimental data or the observations to be stored, the Consultation recommends to the Regional Activity Centres of MED IV and MED V to prepare as quickly and accurately as possible a list of the different experimental parameters, precise conditions of sampling, rough results of analyses or observations compatible with computerization, so as to provide for the best possible utilization of all the results obtained. Such forms will serve to establish the technical data-sheets to be sent to the principal investigators who should undertake to complete them as carefully as possible before returning them to FAO(GFCM).

99. Unpublished data collected by the participants on MED IV and MED V and submitted to FAO(GFCM) or to review meetings attended by participants in MED POL will be considered in their original form as confidential. Results reported by research centres on a semi-annual basis to FAO(GFCM) will be evaluated with the assistance of the appropriate Regional Activity Centre, but will not be otherwise distributed, published or reported in their original form without approval at the relevant research centre.

100. After evaluation of the original data, FAO(GFCM) will provide a generalized appraisal of the results obtained through the pilot projects, as a contribution to the periodic report on the state of pollution of the Mediterranean which will be submitted by UNEP to the Governments of the Mediterranean states.

101. Participants are also encouraged to submit their results to scientific journals acknowledging their involvement in the project and requesting a waiver of copyright by the publisher for UN use of the data (similar to United Kingdom and USA Government prerogatives). The suggested wording for acknowledgement and copyright request is found in document FIR:PM/77/5.

102. The GFCM Reports will continue to contribute to communication between project research centres. In addition, a UNEP newsletter dealing with recent achievements within the Mediterranean Action Plan is being initiated. In the future the GFCM Report will contain an annex citing recent publications of project investigators. UNEP plans to publish annually collected reprints dealing with pollution. It was suggested that FAO publish on its own behalf as information, in the GFCM technical studies and reviews or in technical reports, scientific documents which, for one reason or another, could not be published in the normal scientific journals.

103. A selected bibliography of research relevant to pollution in the Mediterranean, FAO Fish.Tech.Pap., (165) was published and presented. The regional

computed bibliography will be updated and used with the assistance of principal investigators and Regional Activity Centres. Aquatic Science and Fisheries Abstracts (ASFA) can be used as an additional source of information. It was noted that ASFA is being computerized and will be accessible through an information retrieval system. FAO(GFCM) is willing to provide photocopies or microfilms of papers not available to investigators.

FUTURE ORIENTATION AND RECOMMENDATIONS

104. In view of the late start of work and in order to ensure an adequate geographical coverage, the Consultation strongly recommended to extend the duration of pilot projects MED II and MED III to the end of 1978.

105. In order to expand the area covered by MED POL, a joint scientific cruise in the Mediterranean international waters is under investigation and should be planned for 1978. A programme of work should rapidly be outlined by the relevant Specialized Agencies and an organizing committee consisting of competent and experienced scientists. Arrangements should be accelerated so that the cruise may be completed by the end of 1978. Cruise plans and technical aspects will be investigated if and when a sufficient number of participants is interested.

106. It is recommended that the primary objective be training in a framework such as the following:

- (i) planned research with emphasis on oceanographic-environment measurements and their interpretation; sampling, handling and preservation of samples; standard physico-chemical analyses of environmental parameters and of the biomass of pelagic populations; performance of simple algal-growth bioassays and similar investigations;
- (ii) collection of samples of fish, benthic, planktonic and meso-pelagic biota, of water, sediments and floating pollutants for further analyses of hydrocarbons, chlorinated hydrocarbons and heavy metals;
- (iii) execution of a research programme on potential nutritive, eutrophicative or inhabiting properties of surface and deep waters of the Mediterranean along the course of the cruise, using simple algal growth bioassay;
- (iv) continuous observation of the level of visible oil and debris along the course of the cruise.

107. The most suitable ship for the outlined purposes would be a large research vessel with onboard laboratories for basic oceanographic and environmental analyses, with a bioassay laboratory with growth-chambers or free-space for their installation and with adequate oceanographic and sampling gear which can facilitate the above mentioned training and research (basic meteo-oceanographic measurement equipment, standard and large-size water samplers, sediment corers, grabs and dredges, planktonic gear, biologic midwater and demersal trawls, longline and possibly other exploratory fishing gear).

108. Concerning the Unesco paper "The Use of Modelling of Marine Systems in the Framework of UNEP Monitoring and Research Programme", the concept of a model to describe the biogeochemical cycles of important pollutants such as

mercury is acceptable as optional activity within the framework of MED II-V. Special emphasis should be placed on obtaining more information needed for the model (i.e. bioaccumulation rates and retention times of pollutants in order to determine the transfer coefficients between biotic and abiotic compartments; data on the atmospheric and terrestrial input rates of pollutants, as well as on mechanisms and transport rates of the substances from the water column to the sediments). These data will be especially useful to researchers studying the effect of pollutants under MED IV and MED V. The proposed UNEP cruises in international water would be useful in procuring some of these data not readily obtainable from the on-going pilot projects.

109. In connexion with the "black" and "grey" lists used in Protocols referred to in paragraph 63, it was suggested that using the experience available through MED IV and MED V, as well as through the other pilot projects, a critical review be prepared on the properties of substances listed in the "black" and "grey" lists. The review would serve as useful information to the Mediterranean Governments and help them to develop administrative rationale for its application.

110. Specifically it was recommended that:

- (i) relevant components of MED POL in particular MED II, III, IV and V, when operational, be utilized to provide on a regular basis information essential to the establishment of waste discharge control criteria;
- (ii) MED POL research centres, within the confines of present capabilities and support, provide the Mediterranean Governments with characteristics of potentially harmful waste constituents and assess the potential impairment of sea water uses as well as effects on fisheries and marine ecosystems;
- (iii) MED II and MED III research centres (in collaboration with investigators of other pilot projects when appropriate) define
(a) accumulation of these substances in biological materials and sediments, (b) persistence (physical, chemical, biological) in the marine environment - pollution dynamics, (c) susceptibility to physical, chemical and biochemical changes and interaction with other seawater constituents, and (d) environmental transformation of these substances into more harmful chemical forms.

111. This last point, as well as the identification of new contaminants, would be facilitated by the development of a gas chromatography/mass spectrophotometry capability for use of MED POL investigators. MED POL research centres should attempt to accommodate the Intergovernmental Consultation by investigating substances of immediate concern (i.e., organosilicon compounds).

112. It was recommended that principal investigators with chemical background draw their attention to the problems of the chemistry of the contaminants in sea water. Need for investigation of solubility, chemical equilibria, problems of chelation as well as development of suitable analytical techniques has been identified.

113. It is recommended that for the use in MED IV a Manual of bioassay and bioevaluation techniques be compiled. The techniques included should be from published source and their use substantiated by the scientific literature. The techniques included should be critically assessed with their advantages and shortcomings specified. FAO(GFCM)/UNEP should appoint a consultant who will utilize MED POL principal investigators, as well as other experts, in

this task. The Manual should be widely distributed, especially to recently established centres in developing countries.

114. It was recommended that principal investigators in MED IV, when possible, restrict their investigations to a limited number of species meeting the following criteria: (1) readily available and utilized in MED II and MED III, (2) maintenance requirements are understood, and (3) baseline information on parameters of interest exists. Adherence to these criteria should result in an increase of in-depth information on effects of pollutants on representative and ecologically critical or commercially most important species.

115. The proposal for the "Mussel Watch" experiment (document FIR:PM/77/11), as adopted by the Consultation on MED II and MED III (see paragraph 45) was discussed. It was felt that in executing such a programme many problems of biological nature would be faced. It was therefore strongly recommended to involve experienced biologists if and when implementing the "Mussel Watch" experiment and to link it wherever possible to pilot project MED IV.

116. The Consultation further recommended with regard to MED V that:

- (i) every effort be made to facilitate work in zones where studies have not yet been undertaken;
- (ii) reliable methods for pollution evaluation be developed, to this end, the recruitment or development of specialists, particularly of taxonomists, may be necessary;
- (iii) however, in certain conditions and for practical purposes, rapid methods be elaborated (i.e. fouling tests, bioassays and bio-coenological methods);
- (iv) studies on controlled ecosystems be encouraged;
- (v) in the study of ecosystems, development of the link constituted by fish should receive increased attention;
- (vi) a Manual be prepared on guidelines for community and ecosystem studies, preferably through assistance of the Regional Activity Centre.

ADOPTION OF THE REPORT AND CLOSING OF THE SESSION

117. The Report was adopted on 13 May 1977. The Chairman asked Prof. P. Strohal to receive on behalf of the Host Country the appreciation of the Consultation for the excellent technical arrangements. The Consultation was closed on 13 May 1977.

Annex I

AGENDAE

REVIEW OF PILOT PROJECTS MED II
Baseline Studies and Monitoring of Metals, Particularly Mercury and Cadmium,
in Marine Organisms

AND MED III
Baseline Studies and Monitoring of DDT, PCBs
and other Chlorinated Hydrocarbons in Marine Organisms

Dubrovnik, 2-6 May 1977

1. Opening of the Session
2. Adoption of the Agenda and Arrangements for the Session
3. Introductory Statement and General Discussion
 - 3.1 Progress of the Mediterranean Action Plan
 - 3.2 Progress of Pilot Projects MED II and MED III
 - 3.3 General discussion
4. Statistical Considerations in Data Generation and Processing
5. Review of Pilot Project MED II
 - 5.1 Results obtained during the first phase of MED II
 - 5.2 Additional information on pollution of the Mediterranean by metals
 - 5.3 Analytical techniques
 - 5.4 Intercomparison of results
 - 5.5 Assistance programme
6. Review of Pilot Project MED III
 - 6.1 Results obtained during the first phase of MED III
 - 6.2 Additional information on pollution of the Mediterranean by chlorinated hydrocarbons
 - 6.3 Analytical techniques
 - 6.4 Intercomparison of results
 - 6.5 Assistance programme
7. Reporting System
 - 7.1 Exchange, processing and reporting of data
 - 7.2 Information exchange
8. Future Orientation and Recommendations
 - 8.1 Pilot Project MED II
 - 8.2 Pilot Project MED III
9. Other Matters
10. Adoption of the Report
11. Closing of the Session

REVIEW OF PILOT PROJECTS MED IV
Research on the Effects of Pollutants on Marine Organisms
and their Populations

AND MED V
Research on the Effects of Pollutants on Marine Communities and Ecosystems

Dubrovnik, 9-13 May 1977

1. Opening of the Session
2. Adoption of the Agenda
3. Introductory Statement and General Discussion
 - 3.1 Progress of the Mediterranean Action Plan
 - 3.2 Progress of Pilot Projects MED IV and MED V
 - 3.3 General discussion
4. Summary of Review of Pilot Projects MED II and MED III
5. Review of Pilot Project MED IV
 - 5.1 Results obtained during the first phase of MED IV
 - 5.2 Research variables
 - 5.3 Research approaches
 - 5.4 Assistance programme
6. Review of Pilot Project MED V
 - 6.1 Results obtained during the first phase of MED V
 - 6.2 Research variables
 - 6.3 Research approaches
 - 6.4 Assistance programme
7. Reporting System
 - 7.1 Exchange, processing and reporting of data
 - 7.2 Information exchange
8. Future Orientation and Recommendations
 - 8.1 Pilot Project MED IV
 - 8.2 Pilot Project MED V
9. Other Matters
10. Adoption of the Report
11. Closing of the Session

Annex II

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Annex III

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| 2 | Provisional Annotated Agenda |
| 3 | Progress of the Mediterranean Action Plan
by the UNEP Secretariat |
| 4 | Report of the FAO(GFCM)/UNEP Expert Consultation on
the Joint Coordinated Project on Pollution in the
Mediterranean, Rome, 23 June - 4 July 1975 |
| 5 | Joint FAO(GFCM)/UNEP Coordinated Project on Pollution
in the Mediterranean. Report No. 2. GFCM Circular
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| 6 | Statistical Considerations for Baseline Studies and
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by the FAO(GFCM) Secretariat |
| 7 | Compilation of Progress Reports Submitted by Research
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Annex IV

COMPILATION OF PROGRESS REPORTS SUBMITTED BY RESEARCH CENTRES
PARTICIPATING IN THE PILOT PROJECT MED II

Baseline Studies and Monitoring of Metals, Particularly
Mercury and Cadmium in Marine Organisms

1. NORTHWESTERN BASIN

1.1 Spain - Instituto de Investigaciones Pesqueras, Barcelona
(A. Ballester)

Mercury values reported were from analyses of organisms sampled from June 1975 until March 1976. Firm conclusions can only be made after a well designed and executed sampling programme (adequate sample number by size, class and area) and statistical analyses are completed. Mercury values appear to vary with species, habitat, trophic level and growth. High levels reported for *Thunnus thynnus* and *Scylliorhinus canicula* could be explained by individual variation (*S.c.* sample size = 1), trophic level, distribution and/or season (only collected one period in one area), or by size (no small individuals sampled). Individual variation cannot be defined when pooled samples are used. An attempt to relate size to mercury residue has been made. Difference between collecting areas is generally one of the more apparent relationships. This variation between areas is one of the reasons why values from different areas cannot generally be pooled for analysis. As stated above interpretative use of results is most easily justified where the sampling and analysis have been well planned and executed.

1.2 France - I.S.T.P.M., Nantes (Y. Thibaud)

Mercury residues reported for this project were based on June-December 1976 sampling. Values are reported on both wet and dry weight bases. While there appears to be a direct relationship between size (weight) and mercury value in tuna, the relationship is not apparent with the mussel. In mussel there does appear to be considerable variation in residue level between sampling areas.

1.3 France - Laboratoire central d'hygiène alimentaire, Ministère de l'agriculture, Paris (M.G. Cumont)

Samples of more than 700 specimens of *Thunnus thynnus* and *Thunnus alalunga* have been analysed for Hg. The structure of the bluefin tuna population in the Mediterranean has also been studied. The results show values for the Mediterranean that are often 3 to 4 times higher than those for oceanic specimens.

1.4 Italy - Gruppo Ricerca Oceanologica Genova, Istituto di Chimica Generale dell'Università di Genova (R. Capelli)

Data from two projects dealing with the accumulation of heavy metals were reported. An investigation of the distribution of heavy metals (Hg, Cd, Cu, Zn, Pb, Mn, Co, Cr, Ni) in different organs of *Engraulis encrasicolus* was completed as the basis of a thesis. The variation of concentration of metals with collected period (seasonal) and between areas within *Mytilus galloprovincialis* samples has also been completed and submitted for publication.

(i) *Engraulis*

Concentration of heavy metal residues within *Engraulis* seems to show a direct relationship between size and mercury and cadmium concentration. This relationship is not seen with copper, cobalt, nickel or zinc. Zinc gut, gill and muscle levels are apparently higher in the smaller fish. Gill and gut levels are often high indicating perhaps contamination from exposure to the environment, rather than deposition and storage of residues. Liver levels are commonly high for those residues which are absorbed into the blood (mercury, cadmium and copper).

(ii) *Mytilus*

Seasonal sampling of *Mytilus* in the Gulf of La Spezia indicates that between January and June the highest levels of cadmium, copper and zinc were found in February and March. Manganese, cobalt, chromium and nickel had the same trends. There was no apparent relationship between water and tissue concentrations of these metals. *Mytilus* tissue levels did illustrate a pattern that might be related to the movement of contaminated sediment by the current along the northeast shore of the Gulf. These data from the eleven stations emphasize the difficulty of establishing the state of pollution of an area, even a restricted one, such as a port, by considering values from the analysis of samples from a too restricted (number of stations) monitoring effort.

1.5 Italy - Laboratorio per lo Studio della Contaminazione del Mare del CNEN, Fiascherino (A. Brondi/M. Stöppler)

Mercury values for marine organisms sampled by this laboratory between October 1975 and July 1976 were reported. Delay in the installation of the AAS has postponed the analysis of elements other than mercury. Most organisms were analysed individually rather than pooled in order to determine the variation between individuals from the same sample. Mercury determinations have been completed for edible tissue from 275 individuals of 17 species.

Instrument and pretreatment error (variation) were also estimated from replicates of analytical determinations and of pretreatments. The coefficient of variance between instrument determinations ranged from 2.5 to 10%; 10 of 13 replicates showed less than 4% variation. Samples of *Penaeus*, *Sepia* and *Mullus barbatus* all show wide variation between individuals. Samples of *Sepia*, *Octopus* (arms), *Mullus barbatus*, *Sardinia pilchardus* and *Crenilabrus tinca* had relatively high values. Marked differences existed between areas for samples of *Mytilus*, *Mullus* and *Crenilabrus*.

High values were associated with both high trophic level predators and filter feeders with increased consumption of particulate matter with adsorbed metals. Cadmium, copper and lead in coastal waters of the Ligurian and Tyrrhenian Seas were investigated at 225 stations. Water values were high in very clear water, while low in areas rich in algae or suspended particulates. It is suggested that studies should attempt to describe the fate of metals associated with algae, sediment and suspended particulates. Cadmium levels are similar to those reported for the Northwest Basin and the north Adriatic, while lead is an order of magnitude lower than in the north Adriatic.

- 1.6 Italy - Centro di Radiochimica e Analisi per Attivazione del CNR, Istituto di Chimica Generale e Inorganica, Università di Pavia, Pavia (E. Orvini)

Analyses of this research centre have been confined to the determination of mercury, zinc, arsenic and selenium residues in the flesh of tuna collected by the CNEN laboratory at Fiascherino. These few samples show no apparent relationship of the concentration of these elements with either size or area collected.

- 1.7 Italy - Istituto di Anatomia Comparata, Laboratorio di Idrobiologia e Piscicoltura, Università di Siena, Siena (A. Renzoni)

Six elements were monitored in *Mullus barbatus* and *Nephrops norvegicus* from a single site in autumn 1976. There is no relationship between residue concentrations and size within the rather homogeneous groups sampled. Considerable variation exists between individuals, especially with mercury levels which vary by factors of 5 (*Mullus*) and 8 (*Nephrops*). Although both species concentrated mercury, levels in *Mullus* were considerably higher than in *Nephrops*. *Nephrops* concentrated zinc, copper and manganese at levels manifold those found in *Mullus*. Additional data for 1977 were reported.

2. ALGERO PROVENCAL BASIN (ALBORAN SEA TO SICILIAN STRAITS)

- 2.1 Morocco - Institut des pêches maritimes du Maroc (H. Idrissi)

The agreement has been signed with FAO (GFCM) and arrangements for training and delivery of equipment are under way. The sampling programme has recently been initiated and samples are stored deep-frozen. Some earlier results on content of Hg and As in seawater and *Sardina pilchardus* are available.

- 2.2 Algeria - Centre de recherches océanographiques et des pêches, Alger (D. Siblot-Bouteflika)

Preliminary sampling in order to ascertain that the sites were properly chosen have been carried out. *Mullus barbatus* are sampled either on board of trawlers or at landing. At one sampling site *Mytilus galloprovincialis/edulis* is not available and has to be replaced by *Perna perna*. Most equipment has been received and the AAS will soon be installed.

- 2.3 Italy - Istituto di Zoologia e Anatomia Comparata, Università di Messina (L. Moio)

Collection of samples started in December 1976. Sampling areas are located on Sicily and on Calabrian coast as far as possible away from massive pollution sources. The number of sampling sites has been increased as some species are not readily available at all sites. Hg, Cd, Pb and Cu will be determined with AAS, and will be undertaken as soon as the research centre's graphite furnace is installed and the digestion unit is received.

3. ADRIATIC AND IONIAN SEAS

- 3.1 Yugoslavia - Marine Biology Station Portorož, University of Ljubljana (S. Gomišček)

Analyses of marine organisms have not begun since equipment had not

been installed. All preparations (including installation, purchase of chemicals, glassware, standards and literature review) have been performed. Local analysts have been trained and preparations have been made to receive trainees from laboratories of other participating countries.

Although regular analysis will not begin until May 1977, the sampling programme has been under way since September 1976 with additional samples in November 1976 and February 1977 in both clean and polluted areas in the Gulf of Trieste. Samples were quick-frozen and are stored at -20°C . *Mullus barbatus* or *M. surmuletus* are difficult to collect in many sampling localities and it is suggested that *M. barbatus* be replaced with the common and readily obtained *Pagellus erythrinus*. It is also suggested that *Sardina pilchardus*, an important and common pelagic Mediterranean fish, be, together with the tuna, an obligatory monitoring species.

Multielement analysis (Hg, Se, As, Cu, Zn, Pb, Mn, Fe, Cd, Cr, Sb) has been performed on water, sediment, plankton and other selected organisms, using AAS, polarography and NAA in an attempt to understand the distribution of these compounds in the Gulf of Trieste. The most representative picture is given in Hg residues in mussels with normal concentrations 0.01 - 0.04 mg/kg (wet weight), however, those in areas under the influence of the Soča (Isonzo) and Tagliamento Rivers have higher concentrations. Similar trends are apparent in sediments and preliminary analyses of fishes.

3.2 Yugoslavia - Department of Physics and Mathematics, University of Rijeka (A. Ljubičić)

Samples of *Mytilus galloprovincialis* from 4 sites have been collected and analysed for Fe, Cu, Zn, As, Br, Sr and Pb.

3.3 Yugoslavia - Center for Marine Research, "Rudjer Bošković" Institute, Rovinj (M. Branica)

Samples of *Mytilus galloprovincialis* taken in March 1977 from 6 different regions in the North Adriatic have, together with sea water, been analysed for Cd, Pb, Cu and Zn.

3.4 Yugoslavia - Institute for Oceanography and Fisheries, Split (L. Stojanoski)

Analysis of heavy metals in sea water using polarography has started. Samples of *Mytilus galloprovincialis* and *Mullus barbatus* were collected in March 1977 and are stored deep-frozen.

3.5 Malta - The University of Malta, Msida (A. Storace)

Analysis has not begun; it is hoped that specimens collected in September 1976, December 1976 and March/April 1977 will be analysed by summer 1977. Frozen specimens being held are hake (*Merluccius merluccius*), striped mullet (*Mullus barbatus*) and horse mackerel (*Trachurus mediterraneus*). *Mytilus* have been unavailable and collection of *Patella lusitanica* and *P. coerulea* began in March. It is hoped that *Xiphias gladius* might also be available for collection in March/April.

4. AEGEAN AND LEVANT SEAS

4.1 Greece - Nuclear Research Centre "Demokritos", Athens (A.P. Grimanis/C. Papadopoulou)

Mercury, cadmium and zinc were determined in *Mullus barbatus*, *Parapenaeus longirostris*, *Mytilus galloprovincialis* and *Xiphias gladius* by neutron activation analysis. Specimens of *M. barbatus* were collected from three stations in the Saronikos Gulf during winter 1975-76, spring, summer and autumn 1976. Specimens of *P. longirostris* and *M. galloprovincialis* were collected from one station during winter 1975-76 and summer 1976. All cadmium values in *M. barbatus* were found to be less than 0.2 µg/g dry weight.

4.2 Greece - Institute of Oceanographic and Fisheries Research, Athens (V. Voutsinou)

Cadmium was determined in *Mullus barbatus*, *Parapenaeus longirostris* and *Mytilus galloprovincialis* by atomic absorption spectrophotometry. Samples were collected seasonally in the Saronikos Gulf from autumn 1975 to winter 1976. Most cadmium values for *M. barbatus* and *P. longirostris* were found to be less than 0.2 µg/g dry weight while the values for *M. galloprovincialis* showed a greater variation.

4.3 Greece - General State Chemical Laboratories, Athens (D.G. Marketos)

Mercury was determined in *Mullus barbatus* by atomic absorption spectrophotometry. Samples were collected from three sampling stations in the Saronikos Gulf in autumn 1976. Preliminary results for total Hg was reported.

4.4 Greece - Department of Food Hygiene, University of Thessaloniki (A.G. Panetsos)

Mercury levels in autumn 1975 and throughout 1976 for *Mytilus galloprovincialis* showed a wide variation. *Mullus barbatus* analyses were reported for 3 stations for spring and winter collections. They contained very low levels. Twenty eight *Thunnus thynnus* were analysed from 2 sampling stations - one March and one September collection. These values were also relatively low.

4.5 Greece - Laboratory of Analytical Chemistry, University of Thessaloniki (G. Vasilikiotis)

Samples of *Mullus barbatus*, *Thunnus thynnus* and *Mytilus galloprovincialis* collected at 7 stations from September 1975 until December 1976 were analysed in duplicate for mercury, cadmium and lead. Most mercury values in *Mytilus* were low (<0.1 mg/kg) with the highest below established standards. Levels of mercury in *Mullus* were comparable to those in *Mytilus*. *Thunnus* values were also low, with no values as high as 0.1 mg/kg. Cadmium values were similar to those for mercury.

4.6 Turkey - Department of Marine Sciences, Middle-East Technical University, Ankara (T.I. Balkas)

Analytical results for 6 specimens of *Mytilus* sp. collected at Gemlik on the Sea of Marmara in August 1976 and pooled samples of red mullet, shrimp, crab collected in the vicinity of Mersin have been reported. The

species, number of individuals, sizes or date of collection were not specified. All levels were relatively low with cadmium and copper showing an apparent direct relationship between concentration and specimen size.

4.7 Turkey - Hydrobiological Institute, University of Istanbul
(I. Artüz)

The agreement with FAO(GFCM) has recently been signed. Collection of samples which are stored deep frozen has started. Analyses will start when equipment has been installed.

4.8 Turkey - Hydrobiological Institute, Ege University, Izmir
(H. Uysal)

The agreement with FAO(GFCM) was signed in end of April. Preliminary collection of *Mytilus galloprovincialis*, *Mullus barbatus*, *Mugil cephalus* and *Carcinus mediterraneus* at the sampling sites has been carried out. Analyses will start when the AAS has been installed.

4.9 Turkey - Ministry for Food, Agriculture and Animal Husbandry,
General Direction of Aquatic Products, Ankara
(A.M. Atahan)

Collection of samples which are stored has started. The analytical work will be carried out by the Marine Science Department METU, Ankara.

4.10 Cyprus - Fisheries Department, Nicosia (L. Nicolaidou)

Sampling started in September 1976. *Mullus barbatus*, *Xiphias gladius* and *Patella* spp. Samples are stored deep-frozen awaiting the completion of training and delivery of equipment.

4.11 Lebanon - Marine Research Centre - NCSR, Beirut
(H.H. Kouyoumjian)

Due to the situation in Lebanon the work could not be initiated during 1976. The agreement with FAO(GFCM) has been sent to the centre for signature. Preliminary surveys reveal that there might be some difficulties in obtaining certain species.

4.12 Israel - Israel Oceanographic and Limnological Research Ltd.,
Haifa (O. Oren)

The agreement with FAO(GFCM) has been signed and the AAS has been installed and is operational. The analytical work has recently started and the institute has participated in the intercalibration exercise. As *Mytilus galloprovincialis* is not available it has been substituted by *Donax* sp. Samples have been collected since October 1975.

4.13 Egypt - Institute of Oceanography and Fisheries, Alexandria
(H.I. Emara)

The agreement with FAO(GFCM) has been signed and the principal investigator will soon receive training in another participating research centre. The AAS and other equipment will be delivered following the completion of the training. Some difficulties may be encountered in obtaining the required species and as one substitute has been suggested *Sardinella* spp.

Annex V

COMPILATION OF PROGRESS REPORTS SUBMITTED BY RESEARCH CENTRES
PARTICIPATING IN THE PILOT PROJECT MED III

Baseline Studies and Monitoring of DDT, PCBs and other
Chlorinated Hydrocarbons in Marine Organisms

1. NORTHWESTERN BASIN

1.1 Spain - Instituto de Investigaciones Pesqueras, Barcelona
(J.M. Franco)

Intensive sampling of four species (*Mytilus edulis*, *Carcinus mediterraneus*, *Mullus barbatus* and *Sardina pilchardus*) in the Castellón and Barcelona areas was conducted in 1976. Seasonal evaluation was facilitated by five Castellón sampling periods.

In *Mullus barbatus* there appears to be a clear seasonal pattern of chlorinated hydrocarbon accumulation. In Castellón *Mullus* all chlorinated hydrocarbons reached an annual peak in December/January which decreased to a low in September before high levels again appeared in December fish. In the Castellón region the high levels in December/January can be related to the higher fat content of *Mullus* during that period. Barcelona *Mullus* have much higher residues, with the highest values at Montgat. DDE values for *Sardina* in the Castellón area followed the same seasonal pattern shown by *Mullus*, however this was not seen for the other chlorinated hydrocarbon residues because of their elevated levels in the June sample. Here again fat analyses do nothing to clarify the data interpretation. *Sardina*, as with *Mullus*, had higher concentrations of chlorinated hydrocarbon in the Barcelona area, especially PCBs at Montgat.

Mytilus and *Carcinus* results are more complex. Although DDT and its metabolites are present in lower levels than the fishes, PCB levels are uniformly high for all areas in *Carcinus*. In *Mytilus* while DDT values decreased to their lowest values in June, the PCB values increased at Castellón over this period. Seasonal differences in the accumulation and metabolism of these compounds may become clear after further sampling and analyses.

1.2 France - Institut scientifique et technique des pêches maritimes,
Nantes (C. Alzieu)

Chlorinated hydrocarbon analyses were performed on portions of the *Mytilus galloprovincialis* and *Mullus barbatus* samples utilized for mercury analyses. Relatively high PCB levels were analysed. Chlorinated hydrocarbon levels in Leucate Lagoon were notably lower than at other sampling stations.

2. ALGERO-PROVENCAL BASIN (ALBORAN SEA TO SICILIAN STRAITS)

No results were reported for this region. Morocco and Tunisia research centres will be analysing samples from this area in the coming year.

3. ADRIATIC AND IONIAN SEAS

3.1 Yugoslavia - Biological Institute, Dubrovnik (T. Gamulin)

Results of monitoring activities at three stations were reported. PCB levels in net zooplankton and *Mullus surmuletus* are the most notable results. Other chlorinated hydrocarbon values reported were relatively low.

3.2 Yugoslavia - Marine Biology Station Portorož, University of Ljubljana (J. Cencelj)

Previous unpublished results, obtained during 1974-76 were reported. Concentrations of DDT and lindane in sediments and zooplankton from the open waters of the whole Adriatic as well as in fish and molluscs from the north Adriatic were presented. A limited number of PCB analyses had also been performed. No PCBs and only trace amounts of pp-DDT were found in sediments of the open Adriatic. Close to sewage discharges relatively high levels of DDT were found. The GC provided by FAO(GFCM) has been installed and is operational. Preliminary analysis of DDT, dieldrin and PCBs in *Mytilus galloprovincialis* has been performed.

3.3 Yugoslavia - Institute of Oceanography and Fisheries, Split (T. Vučetić)

Sampling started in March 1977 at three sampling stations. *Mullus barbatus*, *Mutilus galloprovincialis*, *Portunus depurator*, *Pachygrapsus marmoratus*, *Xanto hydrophilus*, zooplankton and sediments were sampled. Some analyses were already performed in the "Rudjer Bošković" Institute.

3.4 Yugoslavia - Center for Marine Research, "Rudjer Bošković" Institute, Rovinj (N. Smolaka)

Samples have been collected and the GC is after some delay operational. The analyses will soon be performed.

3.5 Italy - Istituto di Biologia del Mare del CNR, Venezia (V.U. Fossato)

Mytilus sp., *Carcinus mediterraneus* and *Mullus barbatus* were monitored for chlorinated hydrocarbon residues in the Gulf of Venice and near Ancona in 1976. Results indicate that PCB residues are the most important at both stations. BHC, aldrin, dieldrin, DDT and its metabolites were also found in the samples. In this group DDT was the most abundant residue in the filter-feeding *Mytilus* and sediment-feeding *Mullus*, while its metabolite DDE was more abundant in *Carcinus*. The PCB Arochlor 1260 was not reported in *Mytilus* samples, although present in all other samples. Arochlor 1260 and 1254 were present in approximately equal amounts in *Mullus*, while in *Carcinus* the ratio was 1:3. In the *Mullus* sample collected near La Spezia in February 1976 Arochlor 1260 concentration was more than 7 times greater than the 1254 content. Levels in this sample and in the tuna from near Trapani are more than double those found in Gulf of Venice samples.

3.6 Malta - The University of Malta, Msida (J.V. Bannister)

Samples collected in September 1976 were analysed while visiting the Istituto di Biologia del Mare CNR (Venezia). Levels in all samples were relatively low, especially those from *Merluccius*.

4. AEGEAN AND LEVANT SEAS

4.1 Greece - Department of Food Hygiene, University of Thessaloniki
(A.G. Panetsos)

DDT, aldrin and PCBs were determined in *Mytilus galloprovincialis*, *Mullus barbatus* and *Thunnus thynnus* with gas chromatography. Samples were collected from two sampling stations in Thermaikos Gulf, one in Strymonikos Gulf and four in Kavala Gulf during autumn 1975 to winter 1976. The preliminary results were reported.

4.2 Turkey - Middle-East Technical University, Ankara (T. Söylemez)

The results for December 1976 samples of shrimp, crab legs and red mullet collected in the vicinity of Mersin have been reported. In addition to the concentrations of chlorinated hydrocarbons, particularly Arochlor 1260, the variation between shrimp samples should be noted.

4.3 Turkey - Hydrobiological Institute, University of Istanbul
(I. Artüz)

The agreement with FAO(GFCM) has recently been signed. Collection of samples which are stored deep-frozen has started. Analyses will start when equipment has been installed and training of principal investigator is completed.

4.4 Turkey - Ministry of Food Agriculture and Animal Husbandry,
General Direction of Aquatic Products, Ankara
(A.M. Atahan)

Collection of samples which are stored has started. The analytical work will be carried out by the Marine Science Department METU, Ankara.

4.5 Lebanon - Marine Research Centre - NCSR, Beirut (H.H. Kouyoumjian)

Due to the situation in Lebanon the work could not be initiated during 1976. The agreement with FAO(GFCM) has been sent to the centre for signature. Preliminary surveys reveal that there might be some difficulties in obtaining certain species.

4.6 Israel - Israel Oceanographic and Limnological Research Ltd,
Haifa (H. Hornung)

The agreement with FAO(GFCM) has been signed and the analytical work will start soon. Some difficulties have however been encountered with the analytical techniques.

4.7 Egypt - Institute of Oceanography and Fisheries, Alexandria
(M.M. Abbas)

The agreement with FAO(GFCM) has been signed and the principal investigator will soon receive training in another participating research centre. The GC and the equipment will be delivered following the completion of the training. Some difficulties may be encountered in obtaining the required species and as substitutes have been suggested *Sardinella* spp and other species of *Mytilus*.

Annex VI

COMPILATION OF PROGRESS REPORTS SUBMITTED BY RESEARCH
CENTRES PARTICIPATING IN THE PILOT PROJECT MED IV

Research on the Effects of Pollutants on Marine
Organisms and their Populations

1. EGYPT - Institute of Oceanography and Fisheries, Alexandria (H.H. Saleh)

The agreement with FAO(GFCM) has been signed and steps have been taken to provide for training and equipment.

The development and maturation of gonads in *Mullus barbatus*, *Sparus auratus*, *Solea* spp. and *Mugil* spp. will be investigated from collections at sampling areas utilized in the pilot projects for monitoring of pollutants and ecosystem studies.

2. FRANCE - Station marine d'Endoume et centre d'océanographie, Marseille
(G. Bellan)

2.1 Toxicity

In the Endoume laboratory the emphasis has been on a definition of methodology. Assays examining differential effects on development in echinoids began in early 1976; results will soon be available. The in vitro methodology for the study of the interaction of pollutants (especially heavy metals and detergents) with ambient parameters (i.e. salinity) using urchin (*Paracentrotus lividus*) larvae is under way; the flow-through system for this programme has only recently become operational. These studies are being completed in close collaboration with other investigations studying recolonization of portable substrates in natural habitat. Static bioassays are being performed to determine the toxicity of salinity and detergents to *Scoelelepis fuliginosa* and *Capitella capitata*, as well as investigation of their potential synergistic action. These results are being evaluated using the statistical methods of Bliss and Life-Markins. Preliminary tests have shown a well defined tolerance limit with little variability in the level initiating mortality.

Several species of polychaetes, molluscs and amphipods will be exposed to various pollutants in the flow-through system, which is capable of varied and controlled salinity of the sea water, providing a changing environment similar to estuarine conditions. This system will improve the ability to evaluate toxicity results based on in vitro and in situ tests. The goal is to improve the relationship between laboratory data and field observations.

2.2 Development, Reproduction, and Population Genetics

The study of the developmental process may reveal sublethal effects of pollution that could seriously reduce community productivity and/or eliminate populations. The time sequence for development of eggs, fertilization, cleavage, gastrulation and larval stages has been determined for *Paracentrotus lividus*. Percent success and frequency of anomalies at each stage have also been determined. Statistical analysis has proven difficult with the method of Prentici (1976) currently being attempted. The results of this effort should be available in spring 1977.

3. GREECE - Phytopathological Institute "Benaki", Athens (R. Fytizas)

The results were presented of a study on the toxicity, of Paraquat for three marine organisms, i.e. a fish (*Mugil cephalus*), a gastropod (*Murex brandaris*) and a decapod (genus *Pagurus*). The study has been divided in two parts. In the first part survival times were determined at levels of 10, 5, 2.5 and 1 ppm and the pathological changes described. The second part dealt with the accumulation capacity of organisms and the herbicide distribution in different tissues and organs of *M. cephalus*. It has been shown that marine organisms are more sensitive to Paraquat than fresh water fish. At a concentration of 10 ppm, *M. cephalus* survival did not exceed one hour; at 1 ppm, maximum survival was 17 days. Decapods were more sensitive to Paraquat than gastropods. Although less vulnerable than *M. cephalus* to acute poisoning, *Pagurus* was more sensitive to repeated exposure.

Histopathological analysis revealed the existence of serious lesions in various organs of *M. cephalus*. In the branchiae, besides changes frequently noticed with other toxic agents, a typical lesion has been observed, i.e. sclerosis of external extremities of branchial cilia. This lesion is similar to that provoked by the same herbicide in the lungs of mammals. When survival time was long enough, large round wounds could be observed on the abdominal skin of fish.

As regards accumulation and distribution of Paraquat, much larger amounts are found in *Pagurus*; this appears to be related to the high sensitivity of this small crustacean to repeated exposure.

In *M. cephalus*, the largest amounts of Paraquat have been observed in the digestive tract and skin; the lowest values have been registered in muscles.

4. GREECE - Zoological Laboratory and Museum, University of Athens
(M. Moraitou-Apostolopoulou)

Amongst the proposed pollutants, Cu^{2+} in the form of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ has initially been selected. The effects of various concentrations of Cu^{2+} on planktonic copepods *Acartia clausi* and *Oithona mediterranea* have been studied. These organisms were first exposed to low concentrations of Cu^{2+} (0.00025 ppm to 0.009 ppm) in order to detect the possible effects of such sublethal doses.

Except for the lowest dose (0.00025 ppm) a decrease in the survival time has been observed under laboratory conditions with a decline of motility and a lowering of the production rate of genital products.

For the time being, copepods are currently tested with higher doses - 0.027 ppm to 0.054 ppm of Cu^{2+} .

5. ISRAEL - Israel Oceanographic and Limnological Research Ltd., Haifa
(T. Shimoni - E. Nevo)

Heterozygosity and genetic variation may increase fitness and provide genetic flexibility to deal with environmental uncertainty. A reduction in heterozygosity, which might result from a less diverse environment (as with increased pollution), may result in a population more susceptible to reduction or elimination by subsequent environmental alteration. Population genetics studies of *Balanus amphitrite* at three stations in the Bay of Haifa have demonstrated reduced heterozygosity accompanying survival in a more polluted environment. Evidence does not allow a conclusion on the effect

of a specific pollutant on these organisms, but rather the effect of a composite of water quality parameters on gene frequencies in their populations.

6. MALTA - University of Malta (L.J. Saliba - J.V. Bannister)

6.1 Toxicity

LC50s were determined for *Arbacia lixula*, *Paracentrotus lividus* and *Palaemon elegans* (newly hatched larvae, product of laboratory breeding) to mercury, cadmium and copper. Twentyfour hour and 48 hour LC50 values for mercury on *Phaeodactylon tricornutum* will be done in late March. "Whenever possible" specimens were collected 24-48 hours prior to the experiments. Static assays were performed in 1.0-1.5 l beakers. Food was withheld during the test period and 3-4 replicae were run on each assay. The mortality data were plotted on log probability paper and analysed by students' T-test or ANOVA. Twentyfour, 48 and 72 h LC50s for mercury (mercuric sulphate) were respectively 1.5, 0.5 and 0.35 mg/l for *Arbacia lixula*. Preliminary data for *Paracentrotus lividus* seem similar. The 24 h LC50s for copper (cupric sodium citrate) were 86 mg/l (23°C) for *Palaemon elegans* larvae. The 48 h LC50 was 25 mg/l at 20 and 23°C. Bioassays will continue in April.

6.2 Physiological and Behavioural Effects

Field collected *Arbacia lixula* and *Monodonta articulata* were exposed to sublethal levels of mercury (mercuric sulfate) in an attempt to define some physiological indications of reduced fitness. Tests were conducted at 0.1-0.5 mg/l Hg⁺⁺ in 1.5 l beakers for *Arbacia* (72 h LC50 0.35 mg/l). These concentrations produced cytolysis and concentration dependent release of pigment. Monitoring of this pigment (echinochrome, spinochrome, or melanin) in the media may provide an evaluation of sublethal exposure. An "adhesion distress syndrome" was observed during other tests at a lower concentration (0.002 mg/l). These experiments are continuing with animals exposed to 0.002 to 0.05 mg/l Hg⁺⁺ and tested with varied "pulls" to measure the effect on adhesion of the tube-feet to the substrate. Early results have been submitted for publication.

Monodonta were exposed to 0.2-1.0 mg/l Hg⁺⁺ in 250 ml beakers and monitored for 24 h periods using a specially developed "aktograph". Effect on activity was concentration dependent and expressed by an increase in emersion time and reduced waterline activity. Exposure to 0.2 mg/l reduced oxygen consumption to nearly 1/3 of the control value with further decrease with increase in exposure concentration. These experiments are also continuing.

6.3 Development, Reproduction and Population Genetics

Mercury studies on the reproduction and development of the algae *Phaeodactylon tricornutum* have recently begun. This algae is being successfully cultured and seawater controls have been evaluated with regard to total number of cells (Coulter counter), total volume, as well as chlorophyll and protein content.

Work has been completed (some published) on effects of some heavy metal salts (Cu, Pb, Zn) on egg hatching, growth rate and acclimation in *Artemia salina*. Similar studies are under way using mercury and cadmium salts.

Preliminary results indicate that both are more toxic than copper and that lower levels must be tested. Inhibition of hatching and inability to acclimate were noted at 0.001 mg/l Hg^{++} and Cd^{++} .

7. SPAIN - Instituto de Investigaciones Pesqueras, Barcelona (R. Establier)

7.1 Toxicity

The 24 h LC50 was determined for mercury, cadmium and copper on larvae of *Penaeus kerathurus*. Values were characterized by great variation which pointed to the need for increased precision and extension of these studies. The larvae originated from 7 different hatches and although variation was great between larval stages, it also existed between identical stages of different hatches. For mercury (methyl mercuric chloride and mercuric chloride), the LC50s ranged from 3.5 to 12.4 for the former and 4.5 to 12.7 $\mu g/l$ for the latter. The values for cadmium (cadmium chloride) were 0.72 to 1.33 mg/l, while the copper (cupric sulphate) values were 63 to 132 $\mu g/l$. The 24 and 48 h LC50 for *Penaeus kerathurus* and *Palaemonetes varians* larvae, juvenile and adults, as well as adult *Sparus auratus* will be determined for mercury, cadmium and copper.

7.2 Pollutant Dynamics

Heavy metal accumulation and both short- and long-term effects in the fishes *Sparus auratus*, *Mugil auratus* and *Halobatrachus didactylus* were studied. Exposure to mercury, cadmium and copper did not exceed 0.1, 1.0 and 3.0 $\mu g/l$ respectively. Water levels of these contaminants were monitored by AAS analysis. The fish were acclimated to the 36% filtered seawater for ten days before exposure. Two-thirds of the water was exchanged every 48 hours; in long-term studies fish were fed two hours before this water change. Fish used for short-term studies were not fed.

Cadmium concentration in *Halobatrachus didactylus* exposed for 96 hours to 50 mg/l was greatest in the intestine (39 $\mu g/kg$), kidney (13) and liver (5); lower levels were found in the blood (1.2) and muscle (0.2).

Mercury concentrations in *Halobatrachus didactylus* exposed for 49 days to 0.1 mg/l were 50.3 $\mu g/kg$ in liver and 9.6 in muscle; 25 and 10 times, respectively, the control values.

Mugil auratus were sampled after 10, 24, 35, 46 and 57 days exposure to 0.1 mg/l mercury. The tissue from 3 fish was pooled for each analysis. Mercury levels in the gill did not increase after the first week's exposure (9 mg/kg, 100 x control values). Other tissues continued to increase in concentration over the 57 day exposure. Muscle levels increased by a factor of 20, to 2.2 mg/kg; by contrast, gut levels increased 70 times, to 20 mg/kg, while liver levels increased by a factor of 500, to over 100 mg/kg.

Sparus auratus exposed to 0.2 mg/kg copper (cupric sulphate) for 77 days had little increase in gill or muscle copper, but intestine values doubled (2.4 mg/kg), as did milt (8.9). Liver values were greater than five times the control value at 20.1 mg/kg.

7.3 Morphology and Histopathology

Histological studies of tissues from fishes - *Sparus auratus*, *Mugil auratus* and *Halobatrachus didactylus* - exposed to mercury, cadmium and

copper were made for possible pathological effects of media contaminated with sublethal levels. Tissues being evaluated were blood, liver, kidney and intestine.

Halobatrachus didactylus exposed to 0.1 mg/l mercury for 49 days (n = 6) were characterized by intestine with hyperchromatism of nuclei and apical cytoplasm and increased thickness of the villi. *Mugil auratus*, exposed for 57 days to 0.1 mg/l Hg^{++} ($HgCl_2$) had livers with vacuolization and modification of paranchymal cords and intestine with thickened epithelium, disorientation of nuclei, vacuolization and increase in cells in the villi.

Halobatrachus were exposed to 50 mg/l cadmium for 96 hours in April, June and September. Although sample size was only 3, the following effects were observed (1) erythrocytes with varied shape, vacuolization, pycnosis and haemoglobin content; (2) intestinal epithelium with altered nuclear orientation, vacuolization, hypochromatism and necrosis; (3) liver with increased reticular trabeculi and nuclei, and (4) kidney tubules with nuclear disorientation, hypochromatism, reduced lumen or dilated and full of amorphous mass (with eosinophils and signs of degeneration).

Sparus auratus exposed to 0.2 mg/l Cu^{++} for 77 days produced disorganization of the epithelium and basal membrane of the intestine, as well as increased epithelium thickness.

Photomicroscopy was utilized with selected tissue sections to record the effects discussed above.

8. TURKEY - Hydrobiological Institute, Ege University, Izmir (H. Uysal)

The agreement with FAO(GFCM) was signed in end of April and the experimental work will soon start. Bioassays for mercury, cadmium, copper and zinc will be done with *Mytilus galloprovincialis*, *Paracentrotus lividus*, *Carcinus mediterraneus* and *Mugil cephalus*. Analytical studies for these experiments will start as soon as the AAS to be provided is operational.

9. TURKEY - Hydrobiological Institute, University of Istanbul (I. Artüz)

The agreement with FAO(GFCM) has recently been signed but the Institute has already started to investigate different marine organisms for toxicity testing. Water samples have been collected from different sources i.e. from papermill and pesticides industry waste water and short-term static tests have been performed with *Trachurus mediterraneus*, *Carcinus mediterraneus* and *Mytilus galloprovincialis*. Other tests could not be performed as equipment was not received. An important difficulty with these experiments was the reaction of the organisms to the different pH levels and salinities in the waste water.

Samples used in the toxicity tests for heavy metals and pesticides are stored deep-frozen until the AAS and GC equipment is operational.

10. YUGOSLAVIA - Center for Marine Research, "Rudjer Bošković" Institute, Rovinj (B. Kurelec)

The philosophy of this laboratory in the study of biological effects of pollutants commences from the rationale that any change in the environment results in a number of measurable alterations of a physiological and biochemical steady state, i.e. that an environmental stressor causes a number of symptoms which constitute the stress syndrome. A measurable change

in the PS (programmed biosyntheses of DNA, RNA, proteins) may indicate such a syndrome of stress; therefore, the processes of the PS may offer a biochemical index of stress.

Fishes metabolize the aryl hydrocarbon benzo(a)pyrene by a microsomal mixed-function oxidase. This aryl hydrocarbon hydroxylase has been induced in the liver and gills of *Salmo trutta* and *Mallotus villosus* by exposure to petroleum (Payne and Penrose, 1975). Measurements of the activity of this enzyme appears to be a good monitor for marine petroleum pollution, as a sublethal effect that can be quantified. Induction of benzo(a)pyrene hydroxylase (BPH) may be related to (1) potential carcinogenic threat to fish of petroleum pollution, (2) the "taint" problem as a means of quantifying exposure to petroleum products, (3) chronic effects on growth, reproduction, behaviour, and their ecological implications.

In an effort to substantiate the utility of this assay for sublethal effects of petroleum pollution, studies were initiated at "Rudjer Bošković" Institute. *Blennius pavo*, a stationary tide-pool fish common in the Mediterranean, *Sardina pilchardus*, a pelagic fish, and *Microcosmos sulcatus*, a benthic protochordate, have been monitored in these investigations.

Laboratory induction of BPH in *Blennius pavo*, collected from unpolluted areas, resulted in maximum elevation of enzyme activity after 14 days. These induced levels were still present 30 days after termination of exposure to petroleum products. Three days' exposure produced increase in BPH activity. Induction appears to be an all-or-none response; a dose response was not demonstrated. BPH activity was not detected in exposed *Microcosmos*.

Field monitoring of BPH activity in *Blennius* from the Rovinj area was correlated with known pollution levels. In these field tests it was felt that enzyme activity might be a reflection of pollution level (concentration dependent). Aromatic pesticides and PCBs may also induce elevation of BPH activity. In addition, changes in nutrition and hormonal balance have been shown to alter levels of activity of microsomal mixed-function oxidases, such as BPH. On the basis of this knowledge the effect of an oil pollution incident in the Northern Adriatic on BPH activity in the liver of Blenniidae was investigated. New Year 1977 an oil spill caused an increase in the BPH activity which reached a peak on the 23rd day (representing a 20-fold increase of the background level), followed by a decrease in activity until a new background level (4 to 5 times the original background) was reached on the 45th day. This new background level has been constant with subsequent sampling (throughout April - 4 months after the accident). This is the first case history of an oil spill followed up by this biochemical monitoring technique - induction of BPH activity.

The investigation of Y-glutamyl cycle enzymes in natural phytoplankton populations and their role in amino-acid transport was also completed in the frame of this pilot project. This work has been published in Comp. Biochem. Physiol., 53B (1976). This enzyme system is thought to have an important role in utilization of dissolved free amino-acids (DFAA). DFAA in Northern Adriatic waters are also being monitored by this laboratory. The correlation of pollution with changes in the Y-glutamyl cycle enzymes of DFAA in sea waters could provide early notice of decreases in primary production cycles of the sea. This enzyme system has been demonstrated in natural populations of nereids and the sponge, *Geodia cydonium*. DFAA represent an especially nutritious source for protein synthesis and constitutes about 5 percent of the sea's dissolved organic matter (DOM). To place this in perspective, it

should be noted that the sea's total organized matter is estimated to be 0.33% of the mass represented by DOM. Experimental work based on these observations will be undertaken in 1977.

The impact on programmed biosynthesis of detergents in the regenerating sponge (*Geodia cydonium*) was yielded in in vitro effects at the lowest treatment level for which effects have been demonstrated. The lowest treatment level, previously demonstrated to produce an in vitro biological effect, was 10 mg/l on enzyme systems (alkaline phosphatase being the most sensitive), 1 mg/l on isolated cells or organs, and 0.1 mg/l on physico-chemical systems. This study, utilizing the uptake of labelled radio precursors by the sponge, demonstrated a decreased uptake in the acid-soluble fraction at 0.1 mg/l, while incorporation into the acid-insoluble fraction was altered at 0.01 mg/l. Alteration in nucleic acid (DNA and RNA), as well as protein content, was observed in the detergent-exposed sponge cultures. Sodium dodecylsulphate (SDS), an anionic detergent, and a 1:1 mixture of two commercial laundry detergents were used at concentrations from 10^{-9} g/ml to 10^{-5} g/ml. The detergents were taken up but not metabolized by the sponge. Commercial detergents were only 10 percent as active as SDS, but demonstrated similar effects.

The effect of lead and zinc on the 5 aminolevulinate dehydrogenase activity in *Mugil capito* was studied on six specimens exposed to a concentration of 500 μ g Pb/l. Results demonstrate a 37% decrease in the ALA-D activity after one week of exposure, 65% decrease after two weeks and stabilisation of the activity during the third and fourth week. Preincubation of blood samples with 10^{-3} M zinc acetate resulted in expected restoring effect on the ALA-D activity.

11. YUGOSLAVIA - Institute for Oceanography and Fisheries, Split
(R. Mužinić)

Preparations have been made for building up continuous-flow equipment for long-term toxicity tests with heavy metals.

Some preliminary observations have been made on the influence of captivity in the sea bream (*Sparus auratus*) and its behaviour under aquarium conditions. Anaesthesia experiments by means of benzocaine and quinaldine were started with the same species to find out the concentrations appropriate for handling the fish.

Some observations on the mortality of juvenile grey mullets (*Mugil* spp.) in aquaria have also been carried out.

Studies on the sublethal effects of lead on the activity of the 5 aminolevulinate dehydrogenase in adult *Scyliorhinus canicula* were started. Standard method was used.

In the in vitro experiments blood of several fish was pooled. A relationship between the enzyme activity and the concentration of the lead acetate was found.

In the in vivo experiments the lead acetate was given by intraperitoneal injections. Each concentration was tested in individual fish. The data show some relation between the lead acetate concentration and the enzyme activity.

12. YUGOSLAVIA - Biological Institute, Dubrovnik (T. Gamulin)

Research is under way on the distribution and bioaccumulation of DDT

and the PCB Arochlor 1254 on laboratory cultures of zooplankton. Introduction of these pollutants is through cultured marine phytoplankton. It is hoped that this work will increase the understanding of some of the problems associated with the investigation of accumulation, metabolism and effects of chlorinated hydrocarbons on marine diatoms. These problems include the very low solubility of these compounds in water and their high adsorption affinity with solid phases.

The results of the investigation of the distribution of DDT and Arochlor 1254 in the experimental system following exposure of phytoplankton have been reported. The distribution of these low solubility compounds seems to be complex and unpredictable in this system. This may indicate the importance of carefully controlling the conditions of introduction of the contaminants into the system. Problems with low yields include volatility and adsorption.

Annex VII

COMPILATION OF PROGRESS REPORTS SUBMITTED BY RESEARCH CENTRES
PARTICIPATING IN THE PILOT PROJECT MED V

Research on the Effects of Pollutants on Marine Communities
and Ecosystems

1. ALGERIA - Centre de recherches océanographiques et des pêches, Algiers
(R. Semroud)

The effects of the untreated sewage (domestic and industrial) of a city of two million inhabitants on the structure and dynamics of biological communities of the Bay of Algiers are being studied. The relatively unpolluted Bay of Bou Ismail, which is more open and only polluted by a few tourist complexes and agriculture, will provide reference values. Emphasis will be on the macrobenthos of soft substrate. Species composition, diversity, density, biomass, production potential and dynamics of populations and communities are being determined. Environmental parameters surveyed in conjunction with benthos analysis include sediment granulometry, salinity, temperature, dissolved oxygen and organic content of sediments. Pollutants are being monitored in an attempt to correlate their levels with community changes (responses). Benthos surveys and mapping began in April 1976 using a small dredge; with the acquisition of an orangepeel bucket sampler, quantitative sampling, essential to begin population dynamics studies, began in January 1977. Since that time, 4 samples have been collected from each of the 7 stations monthly. The bucket sampler has not been effective for sandy substrate and at these stations comparative studies on the relative effectiveness of an aspirator are now underway.

The water of the Bay is often turbid and its odour can be detected up to 2 km from the coast, especially in the SE portion of the Bay. *Audouinia tentaculata* and the molluscs *Cardium* and *Venus* serve as good indicators of pollution level. Comparative growth studies are being performed with populations of these molluscs from various project stations.

Statistical treatment of the data includes calculation of abundance, dominance, density and biomass, as well as some indices and coefficients of diversity and affinity. Descriptions of growth, mortality and production utilize the equation of Van Bertalanffy, Ford-Walford method and the diagram of Allen. Samples relevant to population dynamics have not been completely processed.

Cartography of the Bay of Algiers, based on 80 stations between 5 and 100 m, provides the first opportunity for the evaluation of pollution effects. In mid-bay, at depths from 0-20 m, the substrate is fine sand the dominant species are *Owenia fusiformis*, *Cardium tuberculatum*, *Spisula subtruncata* and *Macra mactra*. At the same depth, closer to the city, a muddy-sand substrate contains *Owenia fusiformis*, *Audouinia tentaculata*, *Diopatra neapolitana* and *Aonides oxycephala*. In the SE portion of the Bay (across the city) in the sandy mud between the rocks on the bottom at 10-15 m *Owenia fusiformis*, *Amphiura chiajei*, *Nephtys hystericis* and *Sternaspis scutata* predominate. More mud is found as the distant Cape Matifou, on the far side of the Bay, is approached. Detritus is common in the depths around the Cape. Northwest of the Port, at depths of 0-20 m, the substrate is coarse sand. From 20-50 m it is progressively more muddy. At some stations at 50 m, where the mud is reduced, *Audouinia tentaculata* becomes more abundant. Most of the deeper

areas are pure mud and characterized by rather homogeneous communities of *Sternaspis scutata*, *Alpheus glaber*, *Gonoplax rhomboides* and *Nephtys hystericis*.

The influence of pollution in the littoral zone, where hydrodynamics reduce the pollutants, was not clearly apparent. Influence at depths and in protected coastal areas was more clear; sedimentation could be correlated with *Audouinia tentaculata* abundance. In the deeper areas of the Bay communities were represented by a relatively small number of species (*Sternaspis scutata* being dominant in the mud).

2. CYPRUS - Fisheries Department, Nicosia (A. Demetropoulos)

An effort is being made to define the effects of pollution on the ecology of Limassol Bay, especially the benthic communities. Limassol Bay contains two commercial ports, a town of 65 000 and light industry [a slaughterhouse and 7 beverage factories (soft drink, wine, spirit and brewery)]. All wastes are discharged untreated into the bay. Unpolluted Episkopi Bay will be studied for reference values.

Seasonal samples for oceanographic, pollutant, effluent, fishes and sediment and benthos will be collected. Oceanographic values being collected are temperature, dissolved oxygen, salinity, transparency, suspended solids, BOD, nitrites, nitrates, phosphate, and sediment organic content and granulometry. The data for February/March has been processed, but not analysed. Effluent monitoring has also been carried out and values vary greatly with time. Measurements include BOD, pH, conductivity, suspended solids, Cd, Cu, Pb, Zn, Hg, Fe, cyanides and chlorides. Processing of the November/December 1976 benthic samples is well in hand; however results are not available in processed form. Problems have been encountered with diversity and low density of benthos. Biomass calculations are being made.

3. EGYPT - Institute of Oceanography and Fisheries, Alexandria
(M.L. El-Hehyawi)

The agreement with FAO(GFCM) has been signed. The collection of samples covers five stations north of Alexandria and in Abu-Gir bay. The selected localities represent different pollution conditions. The analyses of salinity, nutrients, COD, BOD and other parameters was performed. The determination of abundance of 10 zooplankton components including *Euterpina*, copepods, gastropods, echinoderm larvae and eggs and larvae of fish in the surface water layer show that some species were abundant in the localities at significant distances from the sources of pollution. The waters adjacent to these sources were noticed to contain low numbers of certain species or certain species were almost absent. For example, in early spring 1977 the copepods in the localities influenced by petroleum hydrocarbons were one third of their abundance in the localities influenced by the paper mill while the fish eggs were about five times higher. In both cases the abundance was much lower than in the unpolluted localities.

Some difficulties have been encountered due to lack of some equipment which, however, will be provided soon.

4. FRANCE - Station marine d'Endoume et centre d'océanographie, Marseille

4.1 Benthic studies (D. Bellan-Santini)

Studies of benthic communities and ecosystems are not only time-consuming in the collection and processing of samples and data, but are also

dependent on the passage of time for both temporal and spatial interpretations of the data. Although the studies of this laboratory span a 17-year period, data from areas under investigation in pilot project MED V were begun in late 1975 and early 1976, and interpretable results are not anticipated before 1978.

4.1.1 Hard substrate

Hard substrate analysis is underway in the polluted (domestic, thermal and industrial) Gulf of Fos with comparison of different types of contamination at two depths - less than 3 m and 3-10 m. Analysis of hard substrate benthos from 0-3 m has resulted in a thesis the conclusions of which follow. This habitat type in the Gulf of Fos can be divided into communities associated with degrees of pollution and different dominant organisms. *Cystoseira stricta* is dominant in relatively pure water; *Mytilus galloprovincialis* and *Corallina mediterranea* are common in moderately polluted areas, and *Ulva rigida* in heavily polluted waters.

In the *Cystoseira stricta* community a gradation exists in species composition between the Cape Couronne station and Point Daunelle inside the Gulf. The absence of pure water species (*Hyale schmidti*, *Stenothoe spinimana*, *Caprella liparotensis* and *Ischironome lacazei*) contributes to a decrease in species diversity at Point Daunelle, where the most pollution-tolerant *Jassa falcata* and *Dynamene edwardsi* are found. There is also a decrease in concretion of the substrate. The disappearance of some species (such as *Hyale camptonyx* and *Miniacina miniacea*, which contribute to the character of the substrate) at Cape Couronne accompanies increasing pollution at this station. *Hyale schmidti*, *Caprella liparotensis* and *Jassa falcata* are crustaceans that appear to increase with initial stages of pollution. It would be of interest to verify this with pilot project MED II and MED III results.

The *Mytilus galloprovincialis* and *Corallina mediterranea* communities, while quantitatively similar with regard to number of species collected, differ in relative importance of molluscs, polychaetes and crustaceans. In the *Corallina mediterranea* community crustaceans (*Leptochelia dubia*) are increasing while molluscs and polychaetes have decreased in comparison to the *Mytilus galloprovincialis* community. Pollution appears less important in that portion of the habitat occupied by the *Corallina mediterranea* community. In the moderately polluted environment species equilibrium appears to be easily shifted to better adapted species by the presence of a new source of pollution. This phenomenon is verified by the following example. The *Corallina mediterranea* community exposed to the thermal effluent of the central E.D.F. of Martigues Ponteau has experienced a decrease in number of species present, while the population of *Leptochelia dubia* and *Platynereis dumerilii* have increased. Under the influence of this thermal effluent the normal annual population cycles (characterized by increase in June) of *Mytilus galloprovincialis* and *Corallina mediterranea* are lost. The more heavily polluted waters, containing the *Ulva rigida* community, are characterized by both qualitative and quantitative impoverishment of the photophilic algal components. Composition is limited to those most tolerant of pollution and may show pronounced seasonal fluctuations (i.e. *Platynereis dumerilii* increase in May).

Generally, species distribution in the Gulf of Fos seems correlated with the degree of pollution, with a marked decrease in the number of species with the progression from uncontaminated to polluted waters. Crustaceans are most numerous in unpolluted waters (71% of the community at Cape Couronne and 56% at Point Daunelle) and are the first species to decrease with pollution. Molluscs become more common in moderately polluted areas (37% and 54%).

As pollution increases molluscs also decrease and the polychaetes become most numerous (85%). The calculation of Margalef diversity indices demonstrates an inverse relationship between species diversity and pollution. The Sander's degree of affinity supports the relationship between polluted stations which result from increases in the more pollution-tolerant *Leptochelia dubia*, *Mytilus galloprovincialis* and *Platynereis dumerilii*.

The analysis of hard substrate benthos at depths greater than 3 m was begun in 1977. Early results seem to show the increased importance of algal populations in polluted areas with decreases in animal populations.

4.1.2 Semi-hard substrate

Sessile and motile benthos in *Posidonia* communities of semi-hard substrate are being compared in unpolluted and polluted areas. These investigations began in autumn 1976; however, the first results have not yet been evaluated.

4.1.3 Soft substrate

Soft substrates are under study in areas influenced by:

- (a) the introduction of fresh water and desalinization (commercial and natural), together with domestic and industrial pollution in the Berre Lagoon;
- (b) urban sewage (primarily domestic) - spatial and temporal study in the anchorage of Marseille and a part of Cortiou Bay (Cassis), which includes three lines of investigation:
 - (i) the impact of the waste water of one million people,
 - (ii) the impact of developed beaches, and
 - (iii) the influence of sedimentation;
- (c) the colonization of mixed substrate deposits resulting from dredge activities.

Monthly samples from Berre Lagoon where soft substrate benthos is influenced by the introduction of fresh water are being classified and an evaluation of the results will not be available before 1978. Pre-project results in this area have been published by Bellan and Stora (1976 and 1976a).

Sludge and sediment deposits are filling the southern portion of the Bay of Marseille. The effects of this deposition on community structure is under study in two areas of increasing pollution - the Archipelago of Riou and the Bay of Cassis. Classification and evaluation are well under way. Classification of samples in areas receiving dredge spoils is also under way.

4.1.4 Conclusions

Final conclusions will be based on the entire structure of this project, as well as drawing on the accumulated background of 17 years of benthic study in the area. It is hoped that this background, together with parallel studies in relatively unpolluted environments will allow differentiation of natural variation with time from the effects of pollution. Simultaneous to the field collections, laboratory toxicological experiments and in situ field observations of experimental organisms will be carried out in an effort to increase the knowledge available for the formulation of

conclusions on the effects of municipal and industrial pollution on benthic communities.

4.2 Neritic zone studies (F. Blanc/M. Leveau)

The neritic zone SE of Marseille receives from this urban areas untreated domestic and industrial waste waters which form an extensive sheet of polluted water moving to the east or west (back toward the Bay of Marseille), depending on prevailing current and winds. Both of these areas are used extensively by summer bathers and their condition is important to the condition of local fisheries as well. During the first phase of the study surface water samples will be taken at 40 stations. A large number of measurements will be done in situ, including chemical analyses, determination and enumeration of plankton composition and bacterial counts. Cartography of these measurements will graphically present the relationships of pollutants (as well as some of their physical, chemical and biological effects), and the evolution of the pollutant dilution in space. In addition, the levels of nutrient salts will be useful in the evaluation of the trophic resources of the area. The structure of planktonic communities, their diversity and relationships with pollutants will contribute to an understanding of the local effects of pollution and eutrophication.

The first samples and measurements will be completed in April/May 1977. A follow-up study will be conducted during 10 days in September/October 1977, the first 5-6 days of which will be devoted to obtaining a better understanding of a ecosystem structure. Initial phases of the investigation will provide data on physical and chemical parameters [salinity, temperature, turbidity, seston, dissolved oxygen, nutritive elements (P-PO₄, N-NO₃, N-NO₂, N-NH₄ and Si-SiO₂)], biological parameters [bacterial counts, phytoplankton diversity indices, chlorophyll a and phaeophytin, adenylates (ATP, ADP, AMP), organic carbon and zooplankton] and pollutants [aromatic and total hydrocarbons, phenols, detergents, heavy metal (cadmium, zinc, copper and lead)].

Statistical methods will be used to define the specific structure (spatial and temporal associations and interactions with pollutants) of the planktonic community.

5. GREECE - Institute of Oceanographic and Fisheries Research, Athens (C. Bogdanos)

Sampling of macrozoobenthos at two areas of the north Saronikos Gulf was completed in March 1977. One area is the site of the sewage outfall of a large metropolitan area; the other is the probable site of a future outfall. Parameters measured were diversity, biomass, density, abundance, as well as grain size, organic carbon content and hydrogen sulfide concentration of the sediment. Temperature, salinity, nutrients, and dissolved oxygen also taken into account.

Capitella capitata were the most abundant organisms in the area of the outfall sludge field. Few or no other species were present. The clean zone was characteristic of an Eastern Mediterranean oligotrophic habitat. The survey of this clean site provides background data for studies of succession after the outfall begins dumping.

6. GREECE - Zoological Laboratory and Museum, University of Athens
(C.E. Vamvakas)

Six sites have been selected for the study of fouling communities. One in the Pireus harbour and five in the area near Lavrion harbour, southeast of the Attica peninsula, in depth from 1 to 10 m. Besides general pollution, heated water from an energy plant, mining dust and phosphorus from a match factory are influencing these sites near Lavrion harbour.

The biofouling panels are made of polyvinyl chloride or of asbestos and wood. They will normally be changed by scuba divers every month, and during summertime every fortnight. There is also another series of panels for longer periods.

Environmental parameters measured each month are: temperature, salinity, dissolved oxygen, transparency, phosphates, nitrites, nitrates, ammonia, silicates, pH, suspended matter.

It is hoped that in the same time a study of plankton and of the soft bottom benthic communities will be undertaken in the same area (Lavrion).

7. TURKEY - Hydrobiological Institute, Ege University, Izmir (A. Kocatas)

The agreement was signed only at the end of April 1977. However, the map of benthic communities in Izmir Gulf was established in 1972. Since then, there has been an ongoing industrialization and industrial pollution is now being added to already existing pollution. Industrial and urban wastes are discharged in the gulf without prior treatment.

Main research activities are: (i) physico-chemical factors will be considered, as well as nutrients; (ii) an annual study programme will be undertaken in order to investigate the dynamics of benthic communities on soft and hard substrates.

Qualitative and quantitative sampling has been made early in May 1977. Collected material is now being sorted.

8. YUGOSLAVIA - Biological Institute, Dubrovnik (A. Benović)

The agreement with FAO(GFCM) has been signed and the work has recently started. Results on zooplankton studies as a baseline for the pilot project was however presented. Between 1973 and 1976 several cruises covering the whole Adriatic Sea have been performed.

The biomass of the zooplankton show the highest values in the Northern Adriatic and along the Italian coast, while a decrease along the Yugoslav coast from north to south is obvious. The qualitative composition of zooplankton show that copepods are the most important group in winter while cladocerans show larger amounts in summer. Larvae and other groups are of importance only very locally.

The last four years a great number of samples of micro-zooplankton has been collected. The main group studied is the tintinnides group.

9. YUGOSLAVIA - Marine Biology Station, University of Ljubljana,
Portorož (J. Štirn)

The disruptive effects of municipal sewage on a sea grass (*Cymodocea*

nodosa and *Zosterella nottii*) community are being studied in a controlled environmental experiment. In the Lagoon of Strunjan (Gulf of Triest) two experimental lagoons have been constructed (each 7 x 7 m) containing an undisturbed sea grass community. The city of Piran transports sewage to a 5 m³ settling tank in the vicinity; this tank allows primary treatment of the sewage and a pipeline can deliver controlled amounts of its effluent to the experimental lagoon. This lagoon receives 400 l of this primary-treated sewage once a day during the incoming tide; an amount based on hydraulic and tracer observations and calculated to correspond to the load the Gulf of Koper, a shallow bay, would receive under average conditions, from a city of 50 000 inhabitants. The second lagoon serves as a control and receives no effluent. The experiment will run through two annual cycles, September 1976 until September 1978.

In both experimental and control lagoons, as well as a reference station in the open coastal sea, a large number of environmental measurements will be regularly performed. On a continuous or daily basis, meteorological, pluviometric, tidal and salinity values will be recorded. Bimonthly, 24-hour cyclic observations and measurements of the following will occur:

(1) hydraulic measurements of exchange rates; (2) spectral and quantum recording of solar activity; (3) thermics and evaporation; (4) salinity-density tidal cycling; (5) pH and Eh of water and sediment; (6) alkalinity, total CO₂, Ca, Mg; (7) oxygen, BOD, H₂S; (8) particulate C, P, N, total seston; (9) organic dissolved C, P, N; (10) inorganic NH₃, NO₂, NO₃, PO₄, SiO₂; (11) density of phytoplankton by groups; (12) chlorophyll a, b, c and metabolites; (13) density and biomass of zooplankton by groups; (14) total bacterial counts with fractionation into physiological groups and identification of *Escherichia coli*, *Streptococcus faecalis*, *Clostridium* spp., *Salmonella* spp.; (15) sampling of water, sediments and dominant biota for further analyses of pesticides, PCB, heavy metals, detergents and phenols.

The following ecological phenomena are under continuous study by the pilot project research team:

- (a) Succession, standing crop and productivity at the following community levels (analysis on the species level for dominant or characteristic community members): benthic algae and sea grasses, phytoplankton and tychopelagic diatoms, zooplankton, macrobenthic infauna, meiofauna.
- (b) Recruitment of benthic macrofauna
- (c) Modifications of granulometric, mineralogical and chemical composition of sediments
- (d) Basic microbiological processes, particularly nitrogen cycling
- (e) Modifications of fouling processes
- (f) Modifications of community structure and diversity.

Environmental measurements show a number of important modifications of the ecosystem within the experimental lagoon. Effects resemble the "classical symptoms" of accelerated eutrophication, especially near the bottom, and include increased CO₂, decreased dissolved oxygen, negative Eh, presence of H₂S, increased turbidity and seston. Some parameters normally associated with eutrophication were surprisingly of much less significance than expected; these included nutrient levels, DOC, POC, BOD, total bacterial

counts, faecal coliforms and phytoplankton standing crop. The most remarkable observation has been the absence of any significant phytoplankton or tycho-pelagic bloom in spite of obvious overfertilization by the discharged sewage. Macronutrients have been readily utilized by the massive development of benthic green algae (*Ulva rigida*, *Enteromorpha compressa* and others). The explosive growth of these algae took place during the second month of the experiment effectively extirpating all sea grass vegetation, with its related epiflora and fauna, from the experimentally polluted lagoon. The remaining community, of a quite different type, has been described from similar cases of pollution of natural (non-experimental) communities.

Although observations have been made within infaunal assemblages, fouling community and other ecosystem components, it is too early to provide further interpretative data. A more detailed progress report will be available by the end of 1977.

10. YUGOSLAVIA - Center for Marine Research, "Rudjer Bošković" Institute
Rovinj (D. Zavodnik)

10.1 Phytoplankton/Offshore waters

Offshore waters west of the Istrian peninsula are influenced by the inflow of the Po river into the Northwest Adriatic Sea, as well as effluents from the west Istrian coast and the Rijeka Bay. This area is the most shallow part of the Adriatic with a sandy detritic or detritus ooze bottom. The inflow of fresh water from Italian rivers produce a semiestuarine flowing basin environment with inflowing polluted waters transported to the South Adriatic Sea. Four sampling stations have been established along a transect between Rovinj and the estuary of the Po river. Some hydrological values from past work suggest that the Istrian coast may contribute most of the pollution by metals while the heaviest organic pollution may be contributed by the Italian rivers of the Northwest Adriatic. Investigation of offshore phytoplankton may help to define the relative effects of these sources of pollution.

Hydrographic data, taxonomic analyses of phytoplankton, as well as chlorophyll a and photosynthetic activity of phytoplankton from May and July 1976 offshore cruises were presented. Chlorophyll and photosynthetic values indicate greatest biomass nearest the Po estuary which also correlates with relative nutrient contribution.

10.2 Benthic/Coastal communities

Baseline studies of benthic coastal marine communities in the Northern Adriatic with regard to population dynamics and productivity have been underway since 1960. The present project compares communities at Rijeka exposed to industrial effluents from the city and near an oil terminal with stations near Rovinj exposed to mixed municipal (domestic and industrial) effluent in the Bay of Valdibora and the relatively clean Faborska Bay. This coastal area of West Istria consists of limestone rocks, with sand at 2-10 m and mud at greater depths. Pilot project field work began in late spring and early summer 1976 and benthic sampling has been reported in Rijeka Bay (November 1976). Biomass and photosynthetic activity values have been determined for the eel grass (*Cymodocea nodosa*) communities at Rijeka and Rovinj. The communities of rocky littoral and coastal terrigenous ooze are also being evaluated.

The composition of the rocky littoral communities near Rovinj (West

Istrian coast) and in Rijeka Bay was presented. The dynamics of these communities will be monitored twice a year (summer and winter) at Rijeka and monthly at one of the Rovinj stations. This decision is based on the "identical" composition of the communities, even through both qualitative (i.e. *Chthamalus depressus*, *Littorina neritoides*, *Rivularia atra*, *R. mesenterica*, *Patella lusitanica*, *Hildenbrandtia prototypus*, *Cladophora* spp. and *Ceramium* spp.) and quantitative (i.e. *Catenella apuntia*, *Patella coerulea*, *Lithothamnion lenormandi* and *Fucus virsoides*) differences appear to exist.

Analysis of the sand community was not made; however, in situ measurements of eel grass (*Cymodocea nodosa*) photosynthetic activity were compared in the investigation of possible pollution effects on the community. The biomass of eel grass at Rijeka Bay is 80-100 g/m². Photosynthetic activity in terms of net production in July and November was respectively 0.25 and 0.10 m/O₂/g/h (respectively, temperatures were 22° and 14.5°C and illumination 64 000 and 20 500 luxes). The comparative values at station RO-1 (Rovinj, unpolluted) in the winter were 0.02 ml O₂/g/h, 11.2°C, and 10 000 luxes. The eel grass was unavailable for comparison at the polluted RO-2 station (Rovinj), as it had completely disappeared. Observations of photosynthetic activity will be continued at monthly intervals during 1977 at Rovinj and hopefully seasonally at Rijeka.

Preliminary review of the composition of terrigenous ooze community samples from the Rijeka Bay - both inshore and offshore - was presented. All polychaetes have not yet been identified. The high biomass value (51.47 g wet weight/0.2 m²) at station 7 is attributed to the presence of large specimens of the echinoid *Brissopsis lyrifera*.

11. YUGOSLAVIA - Institute of Oceanography and Fisheries, Split
(T. Pucher-Petković)

Permanent ecological research has been made in a cross section of the central Adriatic, dealing with sea dynamics, hydrological factors, primary production, phytoplankton, zooplankton, ichthyoplankton and planktonic bacteria. This work has started twenty years ago. It has thus been possible to understand the relationship between coastal and offshore ecosystems. Results so far obtained demonstrate the existence in coastal waters of some changes in primary production, community patterns, biomass as well as seasonal fluctuations.

The following preliminary data pertaining to this programme have been collected in March 1977:

- (1) Environmental factors: (i) background hydrographic parameters: temperature, salinity, density, transparency, alkalinity, dissolved oxygen, oxygen saturation; (ii) eutrophication indicators: CO₂, phosphates, nitrates, nitrites, ammonia, silicates; (iii) heavy metals: Zn, Cd, Pb, Cu.
- (2) Plankton: (i) phytoplankton: primary production (C¹⁴), numerical abundance, biomass (pigments), structure; (ii) zooplankton: biomass, qualitative and quantitative structure (main groups), especially copepods; (iii) bacteria: biomass of heterotrophic bacteria.
- (3) Benthos: (i) phytobenthos: structure, abundance, biomass; (ii) zoobenthos: structure, abundance, biomass; (iii) ichthyobenthos: structure, abundance, biomass.
- (4) Nekton: (i) plankton stages of small pelagic fish: abundance, distribution; (ii) adult pelagic fish: abundance, population dynamics, distribution.

Annex VIII

ALTERNATIVE SPECIES FOR MONITORING IN
PILOT PROJECTS MED II AND MED III

In the Operational Document for Pilot Projects MED II and MED III, seven species are listed as obligatory species for monitoring and some others were recommended as additional species. During their work some participating laboratories realized the lack of one or the other obligatory species. Therefore the list reproduced below has been prepared proposing suitable replacements, taking into consideration the following parameters: availability of the species, size of animals, commercial importance, identity of ecological niche, level in trophic chain, and possibility for maintenance of the animals under experimental conditions.

OBLIGATORY SPECIES

Mytilus galloprovincialis
or
Mytilus edulis

Parapenaeus longirostris

Carcinus mediterraneus

Mullus barbatus

Thunnus thynnus thynnus
or
Xiphias gladius

ALTERNATIVE SPECIES

Perna perna
Ostrea edulis and other
oyster species

Arca noae
Lithophaga lithophaga

Penaeus kerathurus

Carcinus maenas
Pachygrapsus marmoratus

Mugil cephalus
M. auratus
M. capito

Sarda sarda
Orcynopsis unicolor

M-45

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