MEDITERRANEAN ACTION PLAN
MED POL

UNITED NATIONS ENVIRONMENT PROGRAMME

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

RESEARCH ON THE TOXICITY, PERSISTENCE, BIOACCUMULATION, CARCINOGENICITY AND MUTAGENICITY OF SELECTED SUBSTANCES (ACTIVITY G)

RECHERCHES SUR LA TOXICITE, LA PERSISTANCE, LA BIOACCUMULATION, LA CANCEROGENICITE ET LA MUTAGENICITE DES CERTAINES SUBSTANCES (ACTIVITE G)

FINAL REPORTS ON PROJECTS DEALING WITH TOXICITY (1983-85)
RAPPORTS FINAUX SUR LES PROJETS AYANT TRAIT A LA TOXICITE (1983-85)

MAP Technical Reports Series No. 10

UNEP
Athens, 1987

This volume is the tenth issue of the Mediterranean Action Plan Technical Report Series.

This Series will collect and disseminate selected scientific reports obtained through the implementation of the various MAP components: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Oil Combating Centre.

Ce volume constitue le dixième numéro de la série des Rapports techniques du Plan d'action pour la Méditerranée.

Cette série permettra de rassembler et de diffuser certains des rapports scientifiques établis dans le cadre de la mise en oeuvre des diverses composantes du PAM: Programme de surveillance continue et de recherche en matière de pollution (MED POL), Plan Bleu, Programme d'actions prioritaires, Aires spécialement protégées et Centre régional de lutte contre la pollution par les hydrocarbures.

INTRODUCTION

The United Nations Environment Programme (UNEP) convened an Intergovernmental Meeting on the Protection of the Mediterranean (Barcelona, 28 January - 4 February 1975), which was attended by representatives of 16 States bordering on the Mediterranean Sea. The meeting discussed the various measures necessary for the prevention and control of pollution of the Mediterranean Sea, and concluded by adopting an Action Plan consisting of three substantive components:

- Integrated planning of the development and management of the resources of the Mediterranean Basin (management component);
- Co-ordinated programme for research, monitoring and exchange of information and assessment of the state of pollution and of protection measures (assessment component);
- Framework convention and related protocols with their technical annexes for the protection of the Mediterranean environment (legal component).

All components of the Action Plan are interdependent and provide a framework for comprehensive action to promote both the protection and the continued development of the Mediterranean ecoregion. No component is an end in itself. The Action Plan is intended to assist the Mediterranean Governments in formulating their national policies related to the continuous development and protection of the Mediterranean area and to improve their ability to identify various options for alternative patterns of development and to make choices and appropriate allocations of resources.

MED POL - Phase I (1976-1980)

The Co-ordinated Mediterranean Research and Monitoring Programme (MED POL) was approved as the assessment (scientific/technical) component of the Action Plan.

The general objectives of its pilot phase (MED POL - Phase I), which evolved through a series of expert and intergovernmental meetings, were:

- to formulate and carry out a co-ordinated pollution monitoring and research programme taking into account the goals of the Mediterranean Action Plan and the capabilities of the Mediterranean research centres to participate in it;
- to assist national research centres in developing their capabilities to participate in the programme;
- to analyse the sources, amounts, levels, pathways, trends and effects of pollutants relevant to the Mediterranean Sea;
- to provide the scientific/technical information needed by the Governments of the Mediterranean States and the EEC for the negotiation and implementation of the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols.
- to build up consistent time-series of data on the sources, pathways, levels and effects of pollutants in the Mediterranean Sea and thus to contribute to the scientific knowledge of the Mediterranean Sea.

MED POL - Phase I initially consisted of seven pilot projects (MED POL I - VII), which were later expanded by additional six pilot projects (MED POL VIII - XIII), some of which remained in a conceptual stage only.

MED POL - Phase I was implemented in the period from 1975 to 1980. The large number of national research centres designated by their Governments to participate in MED POL (83 research centres from 15 Mediterranean States and the EEC), the diversity of the programme and its geographic coverage, the impressive number of Mediterranean scientists and technicians (about 200) and the number of co-operating agencies and supporting organizations involved in it, qualifies MED POL as certainly one of the largest and most complex co-operative scientific programmes with a specific and well-defined aim ever undertaken in the Mediterranean basin.

The overall co-ordination and guidance for MED POL - Phase I was provided by UNEP, acting as the secretariat of the Mediterranean Action Plan (MAP). Co-operating specialized United Nations Agencies (ECE, UNIDO, FAO, UNESCO, WHO, WMO, IAEA, IOC) were responsible for the technical implementation and day-to-day co-ordination of the work of national research centres participating in the pilot projects.

MED POL - Phase II (1981 - 1990)

The Intergovernmental Review Meeting of Mediterranean Coastal States and First Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution, and its related protocols (Geneva, 5-10 February 1979), having examined the status of MED POL - Phase I, recommended that during the 1979/80 biennium a Long-term pollution monitoring and research programme should be formulated.

Based on the recommendations made at various expert and intergovernmental meetings, a draft Long-term (1981 - 1990) Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II) was formulated by the Secretariat of the Barcelona Convention (UNEP), in co-operation with the United Nations Agencies which were responsible for the technical implementation of MED POL - Phase I, and it was formally approved by the Second Meeting of the Contracting Parties of the Mediterranean Sea against pollution and its related protocols and Intergovernmental Review Meeting of Mediterranean Coastal States of the Action Plan held in Cannes, 2-7 March 1981.

The general long-term objectives of MED POL - Phase II were to further the goals of the Barcelona Convention by assisting the Parties to prevent, abate and combat pollution of the Mediterranean Sea Area and to protect and enhance the marine environment of the Area. The specific objectives were designed to provide, on a continuous basis, the Parties to the Barcelona Convention and its related protocols with:

- information required for the implementation of the Convention and the protocols;

- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;

- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;

- information which could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis;

- periodic assessment of the state of pollution of the Mediterranean Sea.

The monitoring of, and research on, pollutants affecting the Mediterranean marine environment reflects primarily the immediate and long-term requirements of the Barcelona Convention and its protocols, but also takes into account factors needed for the understanding of the relationship between the socio-economic development of the region and the pollution of the Mediterranean Sea.

For this purpose, monitoring was organized on several levels:

- monitoring of sources of pollution providing information on the type and amount of pollutants released directly into the environment;

- monitoring of nearshore areas, including estuaries, under the direct influence of pollutants from identifiable primary (outfalls, discharge and coastal dumping points) or secondary (rivers) sources;

- monitoring of offshore areas (reference areas) providing information on the general trends in the level of pollution in the Mediterranean;

- monitoring of the transport of pollutants to the Mediterranean through the atmosphere, providing additional information on the pollution load reaching the Mediterranean Sea.

Research and study topics included initially in the MED POL - Phase II were:

- development of sampling and analytical techniques for monitoring the sources and levels of pollutants. Testing and harmonization of these methods at the Mediterranean scale and their formulation as reference methods. Priority will be given to the substances listed in the annexes of the Protocol for the prevention of pollution of the Mediterranean Sea by dumping from ships and aircraft and the Protocol for the protection of the Mediterranean Sea against pollution from land-based sources (activity A);

- development of reporting formats required according to the Dumping, Emergency and Land-Based Sources Protocols (activity B);

- formulation of the scientific rationale for the environmental quality criteria to be used in the development of emission standards, standards of use or guidelines for substances listed in annexes I and II of the Land-Based Sources Protocol in accordance with Articles 5, 6 and 7 of that Protocol (activity C);

- epidemiological studies related to the confirmation (or eventual revision) of the proposed environmental quality criteria (standards of use) for bathing waters, shellfish-growing waters and edible marine organisms (activity D);

- development of proposals for guidelines and criteria governing the application of the Land-Based Sources Protocol, as requested in Article 7 of that Protocol (activity E);

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The general long-term objectives of MED POL - Phase II were to further the goals of the Barcelona Convention by assisting the Parties to prevent, abate and combat pollution of the Mediterranean Sea Area and to protect and enhance the marine environment of the Area. The specific objectives were designed to provide, on a continuous basis, the Parties to the Barcelona Convention and its related protocols with:

- information required for the implementation of the Convention and the protocols;
- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;
- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;

INTRODUCTION

Le Programme des Nations Unies pour l'environnement (PNUE) a convoqué une réunion intergouvernementale sur la protection de la Méditerranée (Barcelone, 28 janvier - 4 février 1975) à laquelle ont pris part des représentants de 16 Etats riverains de la mer Méditerranée. La réunion a examiné les diverses mesures nécessaires à la prévention et à la lutte antipollution en mer Méditerranée, et elle s'est conclue sur l'adoption d'un Plan d'action comportant trois éléments fondamentaux:

- Planification intégrée du développement et de la gestion des ressources du bassin méditerranéen (élément "gestion");

- Programme coordonné de surveillance continue, de recherche, d'échange de renseignements et d'évaluation de l'état de la pollution et des mesures de protection (élément "évaluation");

- Convention cadre et protocoles y relatifs avec leurs annexes techniques pour la protection du milieu méditerranéen (élément juridique).

Tous les éléments du Plan d'action étaient interdépendants et fournissaient le cadre d'une action d'ensemble en vue de promouvoir tant la protection que le développement continu de l'écorégion méditerranéenne. Aucun élément ne constituait une fin à lui seul. Le Plan d'action était destiné à aider les gouvernements méditerranéens à formuler leurs politiques nationales en matière de développement continu et de protection de zone de la Méditerranée et à accroître leur faculté d'identifier les diverses options s'offrant pour les schémas de développement, d'arrêter leurs choix et d'y affecter les ressources appropriées.

MED POL - Phase I (1976 - 1980)

Le programme coordonné de surveillance continue et de recherche en matière de pollution de la Méditerranée (MED POL) a été approuvé au titre de l'élément "évaluation" (scientifique/technique) du Plan d'action.

Sa phase pilote (MED POL - Phase I) avait les objectifs généraux ci-dessous, élaborés au cours d'une série de réunions d'experts et de réunions intergouvernementales:

- formuler et exécuter un programme coordonné de surveillance continue et de recherche en matière de pollution en tenant compte des buts du Plan d'action pour la Méditerranée et de l'aptitude des centres de recherche méditerranéens à y participer;

- aider les centres de recherche nationaux à se rendre plus aptes à cette participation;

- étudier les sources, l'étendue, le degré, les parcours, les tendances et les effets des polluants affectant la mer Méditerranée;

- fournir l'information scientifique et technique nécessaire aux gouvernements des pays méditerranéens et à la Communauté économique européenne pour négocier et mettre en oeuvre la Convention pour la protection de la mer Méditerranée contre la pollution et les protocoles y relatifs;

- research on oceanographic processes, with particular emphasis on surface circulation and vertical transport. Needed for the understanding of the distribution of pollutants through the Mediterranean and for the development of contingency plans for cases of emergency (activity F);

- research on the toxicity, persistence, bioaccumulation, carcinogenicity and mutagenicity of selected substances listed in annexes of the Land-Based Sources Protocol and the Dumping Protocol (activity G);

- research on eutrophication and concomitant plankton blooms. Needed to assess the feasibility of alleviating the consequences and damage from such recurring blooms (activity H);

- study of ecosystem modifications in areas influenced by pollutants, and in areas where ecosystem modifications are caused by large-scale coastal or inland engineering activity (activity I);

- effects of thermal discharges on marine and coastal ecosystems, including the study of associated effects (activity J);

- biogeochemical cycle of specific pollutants, particularly those relevant to human health (mercury, lead, survival of pathogens in the Mediterranean Sea, etc.) (activity K);

- study of pollutant-transfer processes (i) at river/sea and air/sea interface, (ii) by sedimentation and (iii) through the straits linking the Mediterranean with other seas (activity L);

As in MED POL - Phase I, the overall co-ordination and guidance for MED POL - Phase II is provided by UNEP as the secretariat of the Mediterranean Action Plan (MAP). Co-operating specialized United Nations Agencies (FAO, UNESCO, WHO, WMO, IAEA, IOC) are responsible for the technical implementation and day-to-day co-ordination of the work of national centres participating in monitoring and research.

The first eight volumes of the MAP Technical Reports Series present the collection of final reports of the Principal Investigators who participated in the relevant pilot projects (MED POL I - MED POL VIII). The ninth volume of the MAP Technical Reports Series is the final report on the implementation of MED POL - Phase I, prepared, primarily, on the basis of individual final reports of the principal investigators with the co-operation of relevant United Nations Agencies (FAO, UNESCO, WHO, WMO, IAEA, IOC).

With the initiation of MED POL - Phase II, 13 research proposals were submitted in the framework of activity G "Research on the toxicity, persistence, bioaccumulation, carcinogenicity and mutagenicity of selected substances. Ten of these projects were finally implemented and completed in 1985. All of them were concerned with the toxicity and bioaccumulation of selected substances.

This tenth volume of the MAP Technical Report Series includes the final reports on the projects which mainly dealt with the effects of oil and oil dispersants on marine organisms. The other final reports on projects will appear in future issues of the Series.

- les renseignements dont elles avaient besoin pour appliquer la Convention et les protocoles;

- des indications et une évaluation de l'efficacité des mesures prises pour prévenir la pollution en application de la Convention et des protocoles;

- des renseignements scientifiques qui pourraient servir à réviser et modifier les dispositions pertinentes de la Convention et des protocoles et à rédiger des protocoles additionnels;

- des informations qui pourraient servir à formuler sur les plans national, bilatéral et multilatéral, les décisions de gestion, respectueuses de l'environnement, qui seraient indispensables à la poursuite du développement socio-économique de la région méditerranéenne;

- une évaluation périodique de l'état de pollution de la mer Méditerranée.

La surveillance continue des polluants affectant le milieu marin de la Méditerranée ainsi que la recherche menée à leur sujet répondent en premier lieu aux prescriptions immédiates et à long terme de la Convention de Barcelona et des protocoles y relatifs, mais elles tiennent également compte des facteurs requis pour la compréhension des relations existant entre le développement socio-économique de la région et la pollution de la mer Méditerranée.

A cette fin, la surveillance continue était organisée à plusieurs niveaux:

- mise au point de techniques d'échantillonnage et d'analyse pour la surveillance des sources et des niveaux de pollution. Essai et harmonisation de ces méthodes à l'échelle méditerranéenne, et formulation de méthodes de référence. Substances figurant sur les listes de priorité des protocoles sur les opérations d'immersion et sur la pollution d'origine tellurique (activité A);

- mise au point de la présentation type des rapports à soumettre en application des protocoles relatifs à l'immersion, à la pollution résultant de situations critiques et à la pollution d'origine tellurique (activité B);

- élaboration des fondements scientifiques des critères de qualité de l'environnement qui serviront à définir des normes d'émission, des normes d'usage ou des directives concernant les substances énumérées dans les annexes I et II du protocole relatif à la pollution d'origine tellurique, conformément aux articles 5, 6 et 7 de ce protocole (activité C);

- études épidémiologiques relatives à la confirmation (ou révision éventuelle) des critères de la qualité de l'environnement (normes d'usage) proposés pour les eaux servant à la baignade, à la culture de coquillages et à l'élevage d'autres organismes marins comestibles (activité D);

- mise au point de projets de directives et de critères régissant l'application du protocole relatif à la pollution d'origine tellurique, conformément à l'article 7 de ce protocole (activité E);

- constituer des séries chronologiques cohérentes de données sur les sources, les cheminements, les degrés et les effets des polluants de la mer Méditerranée et contribuer par là à la connaissance scientifique de cette mer.

La Phase I du MED POL comportait à l'origine sept projets pilotes (MED POL I - VII) auxquels sont venus ultérieurement s'ajouter six autres (MED POL VIII - XIII) dont certains n'en sont restés qu'au stade de la conception.

La Phase I du MED POL a été mise en oeuvre au cours de la période 1975 - 1980. Le grand nombre de centres de recherche nationaux désignés par leurs gouvernements pour participer au MED POL (83 centres de recherche de 15 Etats méditerranéens et de la CEE), la diversité du programme et sa couverture géographique, l'effectif impressionnant de scientifiques et techniciens méditerranéens (environ 200) ainsi que la quantité d'organismes coopérants et d'organisations d'appui qui y étaient engagés permettent sans conteste de caractériser le MED POL comme l'un des programmes de coopération scientifique les plus vastes et les plus complexes, comportant un objectif spécifique et bien défini, qui ai jamais été entrepris dans le bassin méditerranéen.

La coordination et la direction générales de MED POL - Phase I ont été assurées par le PNUE, faisant fonction de secrétariat du Plan d'action pour la Méditerranée (PAM). Les organismes spécialisés coopérants des Nations Unies (CEE - Commission économique pour l'Europe, ONUDI, FAO, UNESCO, OMS, OMM, AIEA, COI) étaient chargés de l'exécution technique et de la coordination quotidienne des travaux des centres de recherche nationaux participant aux projets pilotes.

MED POL - Phase II (1981 - 1990)

La réunion intergouvernementale des Etats riverains de la Méditerranée chargés d'évaluer l'état d'avancement du Plan d'action et première réunion des Parties contactantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux protocoles y relatifs (Genève, 5 - 10 février 1979), ayant examiné la situation de la Phase I du MED POL, a recommandé que, durant la période biennale 1979 - 80, soit formulé un programme à long terme de surveillance continue et de recherche en matière de pollution.

Sur la base des recommandations énoncées lors des diverses réunions d'experts et réunions intergouvernementales, un projet de programme à long terme (1981 - 1990) de surveillance continue et de recherche en matière de pollution (MED POL - Phase II) a été formulé par le secrétariat de la Convention de Barcelone (PNUE), en coopération avec les organismes des Nations Unies chargés de l'exécution technique de MED POL - Phase I, et il a été officiellement approuvé lors de la deuxième réunion des Parties contractantes à la Convention pour la protection de la mer Méditerranée contre la pollution et aux Protocoles y relatifs et réunion intergouvernementale des Etats riverains de la mer Méditerranée chargée d'évaluer l'état d'avancement du Plan d'action, qui s'est tenue à Cannes du 2 au 7 mars 1981.

L'objectif général à long terme de la Phase II du MED POL était de concourir à la réalisation des objectifs de la Convention de Barcelone en aidant les Parties contractantes à prévenir, réduire et combattre la pollution dans la zone de la mer Méditerranée ainsi qu'à protéger et améliorer le milieu marin dans cette zone. Les objectifs particuliers étaient de fournir constamment aux Parties contractantes à la Convention de Barcelone et aux Protocoles y relatifs:

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- recherches sur les processus océaniques, et particulièrement sur la circulation en surface et les déplacements verticaux. Cette information est nécessaire à la connaissance de la répartition des polluants en Méditerranée et à la mise au point de plans pour parer aux situations critiques (activité F);

- recherches sur la toxicité, la persistance, la bioaccumulation et le caractère cancérigène et mutagène de certaines substances énumérées dans les annexes du protocole relatif à la pollution d'origine tellurique et du protocole relatif aux opérations d'immersion (activité G);

- recherches sur l'eutrophisation et les floraisons de plancton qui l'accompagnent. Cette information est nécessaire pour évaluer la possibilité de prévenir les effets et les dégâts causés par ces floraisons périodiques (activité H);

- étude des modifications de l'écosystème dans les zones soumises à l'influence des polluants et dans celles où ces modifications sont dues à d'importantes activités industrielles sur la côte ou à l'intérieur des terres (activité I);

- effets des pollutions thermiques sur les écosystèmes marins et côtiers, y compris l'étude des effets connexes (activité J);

- cycle biogéochimique de certains polluants intéressant particulièrement la santé (mercure, plomb, survie des organismes pathogènes dans la mer Méditerranée, etc.) (activité K);

- étude des processus de transfert des polluants (i) aux points de contact entre les cours d'eau et la mer et entre l'air et la mer, (ii) par sédimentation et (iii) à travers les détroits qui relient la Méditerranée aux mers voisines (activité L).

Comme lors de la Phase I du MED POL, la coordination et la direction générales de la Phase II étaient assurées par le PNUE, par l'intermédiaire du secrétariat du Plan d'action pour la Méditerranée (PAM). Les organismes spécialisés coopérants des Nations Unies (FAO, UNESCO, OMS, OMM, AIEA, COI) étaient chargés de l'exécution technique et de la coordination quotidienne des travaux des centres de recherche nationaux participant au programme de surveillance continue et de recherche.

Les huit premiers volumes de la Série des rapports techniques du PAM rassemblent les rapports finaux de chercheurs responsables qui ont participé aux projets pilotes correspondants (MED POL I - MED POL VIII). Le neuvième volume de cette même Série se compose du rapport final sur la mise en oeuvre de la Phase I du programme MED POL, établi essentiellement sur la base des rapports finaux individuels des chercheurs responsables avec la coopération des organismes compétents des Nations Unies (FAO, UNESCO, OMS, OMM, AIEA, COI).

A l'occasion du lancement de la Phase II du MED POL, 13 propositions de recherche ont été soumises dans le cadre de l'activité G intitulée "Recherches sur la toxicité, la persistance, la bioaccumulation, la cancérogénicité et la mutagénicité de certaines substances". Dix de ces projets ont finalement été exécutés et menés à bon terme en 1985. Ils avaient tous trait à la toxicité et à la bioaccumulation de certaines substances.

Le présent volume, le dixième de la Série des rapports techniques du PAM, comprend les rapports finaux sur les projets traitant principalement des effets de hydrocarbures et des dispersants d'hydrocarbures sur les organismes marins. Les autres rapports finaux sur des projets figureront dans les prochaines publications de la Série.

EFFECTS OF OIL DISPERSANTS ON MARINE ORGANISMS

by

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A B S T R A C T

In order to help assess the likely environmental impact of the use of oil dispersants as an oil pollution control measure, the biological effects of crude oil dispersed with a variety of dispersants were compared to those of mechanically dispersed oil in a number of laboratory investigations. All dispersants used were of the newer generation "low toxicity" type. Both exposures at sea and on the beach were simulated. Chemically dispersed petroleum hydrocarbons (PHC) generally altered the various biological responses monitored in a range of marine organisms, significantly more than the mechanically dispersed PHC. These responses include: decreased growth rates of diatom Phaeodactylum tricornutum; increased mortalities of the limpets Patella caerulea, and Patella rustica, of the sea urchin Paracentrotus lividus and of the shrimp, Palaemon elegans; increased oxygen consumption in P. caerulea, P. rustica, P. lividus and the bivalve Venus verrucosa; decrease in bioadhesion of P. caerulea; increased rates of ammonia excretion as well as a decrease in the physiological stress indices of scope for growth and oxygen: nitrogen in V. verrucosa; interference with the righting behaviour of P. lividus as well as reduced food detection in the hermit crab Clibanarius erythropus. These effects were generally dependent on the concentrations of PHC as well as on the dispersant applied. Many of the investigations were not concluded by the time of this report (July 1985). Further results are expected to clarify and confirm the conclusion presented here.

1. INTRODUCTION

Coastal and Mediterranean waters are often exposed to both short-term and chronic pollution by oil. Though a variety of control methods are in use or have been proposed, the use of chemical dispersants is considered to be the most effective and most common. The scope of the present project was to evaluate the possible lethal and sublethal biological effects of the use of the recent generation of oil dispersants. Although the latest generation of dispersants are considerably less toxic to marine life than the former ones, more information is required on their long-term sublethal effects on the physiology and behaviour of marine forms. Moreover, it is now realized that the investigation of the biological effects of dispersants alone is of little use. In the field, marine organisms are more oftenly exposed to a mixture of oil and dispersant rather than to the dispersant alone (Norton and Franklin, 1980). It is therefore more ecologically realistic to investigate the biological effects of chemically dispersed oils and relate them to those of mechanically dispersed oil.

2. METHODOLOGY

The dispersants investigated were all of the latest "low toxicity" type. They include both concentrates and solvent based dispersants. The concentrates were applied as a 10% dilution in sea water as recommended by the manufacturers, unless otherwise indicated in the text. Kuwait Crude Oil (batch A/102 - supplied by the Kuwait Petroleum Corporation) was used throughout all investigations. Unless otherwise indicated, oil was used fresh. Both dispersants and oil were stored in small containers (volume: 500 ml to 2 l) at 10 °C prior to use.

Petroleum hydrocarbon (PHC) analyses of test media were carried out by extraction with dichloromethane (Spectrosol Grade) which was dried over anhydrous sodium sulphate and then its fluorescence read on a Turner 430 spectroflourimeter at 310 nm excitation and 360 nm emission wavelengths. Calibration was carried out using known weights of fresh Kuwait Crude in dichloromethane and results are expressed in Kuwait Crude Oil equivalents ($\mu g\ l^{-1}$). A wide range of species were experimented on including phytoplankton (Phaeodactylum tricornutum), sublittoral (Venus verrucosa, Paracentrotus lividus, Palaemon elegans) and littoral forms (Patella caerulea, Patella rustica, Clibanarius erythropus). All species were collected from local unpolluted coastal areas, and acclimated in holding tanks supplied from a closed circuit sea water circulating system maintained at 19-20 °C; 37 ppt salinity and light dark cycle 12:12 h.

A wide range of biological responses on exposure to oil, dispersants or oil plus dispersant mixtures were investigated, including: mortality, physiological and behavioural responses. Details of methods are included in Section 3.

3. INVESTIGATIONS

3.1 Phaeodactylum tricornutum (BACILLARIOPHYCAE)

3.1.1 Toxicity of crude oil and crude oil plus dispersant mixtures

Materials and Methods

Phaeodactylum tricornutum (culture batch number 1052/1A obtained from The Culture Centre of Algae and Protozoa, Cambridge, U.K.) was cultured in marine algal maintenance medium (Ward and Parrish, 1982) at 20 °C under continuous 2000 Lux illumination. Two weeks prior to use, the diatoms were transferred to a test medium (Ward and Parrish, 1982) for acclimation. All media and acid washed glassware were sterilized before use. One cm^3 of inoculum from actively growing stock cultures in the test medium were used in the experiments. Starting algal concentrations ranged from 10 to 15 x 10^4 cells cm^{-3}. Cultures were exposed to oil or oil plus dispersant mixtures for 24 h in 125 cm^3 cotton-plugged Erlenmeyer flasks with a total volume of 50 cm^3 test medium at 20 °C under 2000 Lux continuous illumination and continuous agitation being maintained by placing flasks in an upright position on a shaking platform with circular motion of 100 revolutions per minute.

Immediately after inoculation, the experimental flasks were dosed with varying amounts (0.5, 1, 2, 4 and 8 µl) of oil or oil and dispersant (equal volumes) mixtures. Three replicate flasks were used per treatment level as well as for the control. In all cases the experiment for each dispersant was

repeated at least twice. After 24 h, cell concentrations were determined by a haemocytometer (Improved Neubauer Levy Ultra Plane). Quadruple counts were made for each flask. The above experiments were carried out at four different temperatures (12, 16, 20 and 25 °C) for one particular dispersant, experimental temperatures being maintained by placing flasks in a closed circuit water bath on the shaking table.

Results

Levels of PHC in the test mixtures at 20 °C for one particular dispersant are presented in Table I, indicating that concentrations were greater in the presence of dispersant. As expected, there were significant losses in the test PHC concentrations over the 24 h exposure period, especially at the lower range of concentrations. Such losses were less pronounced in the presence of the dispersant.

Table I

Phaeodactylum tricornutum levels of PHC in ppm in test solutions at 0 h and at 24 h of exposure at 20 °C

Nominal concentrations in µl per 50ml		0 h	24 h
Oil	Dispersant		
0.5	0	2.72	1.7
1	0	3.77	2.04
2	0	22.53	3.36
4	0	29.05	14.95
8	0	37.85	29.05
0.5	0.5	3.17	1.87
1	1	14.90	2.57
2	2	26.40	7.03
4	4	60.75	21.12
8	8	144.40	47.56

The mean 24 h cell counts for each treatment level as expressed in mean percent of control, are presented in Figs. 1 and 2. Any differences between means of pairs of oil and oil plus dispersant mixtures were statistically analysed by Duncan's multiple range test (Alder and Roessler, 1977). Generally, a wide range of variability in response to exposure was recorded. At the lower test concentration, no consistent toxic effect on the growth rate could be detected for any of the dispersants investigated. Stimulation of algal growth in some cases was recorded. Similar algal growth stimulations by PHC were reported by other authors (eg. Kauss and Hutchinson, 1975). At the higher test concentrations, three of the dispersants significantly increased the toxic effect of oil on algal growth (at $P = 0.05$). Moreover this increase in toxicity was more significant at higher temperatures as seen from data presented in Fig. 2, even at lower concentrations of PHC.

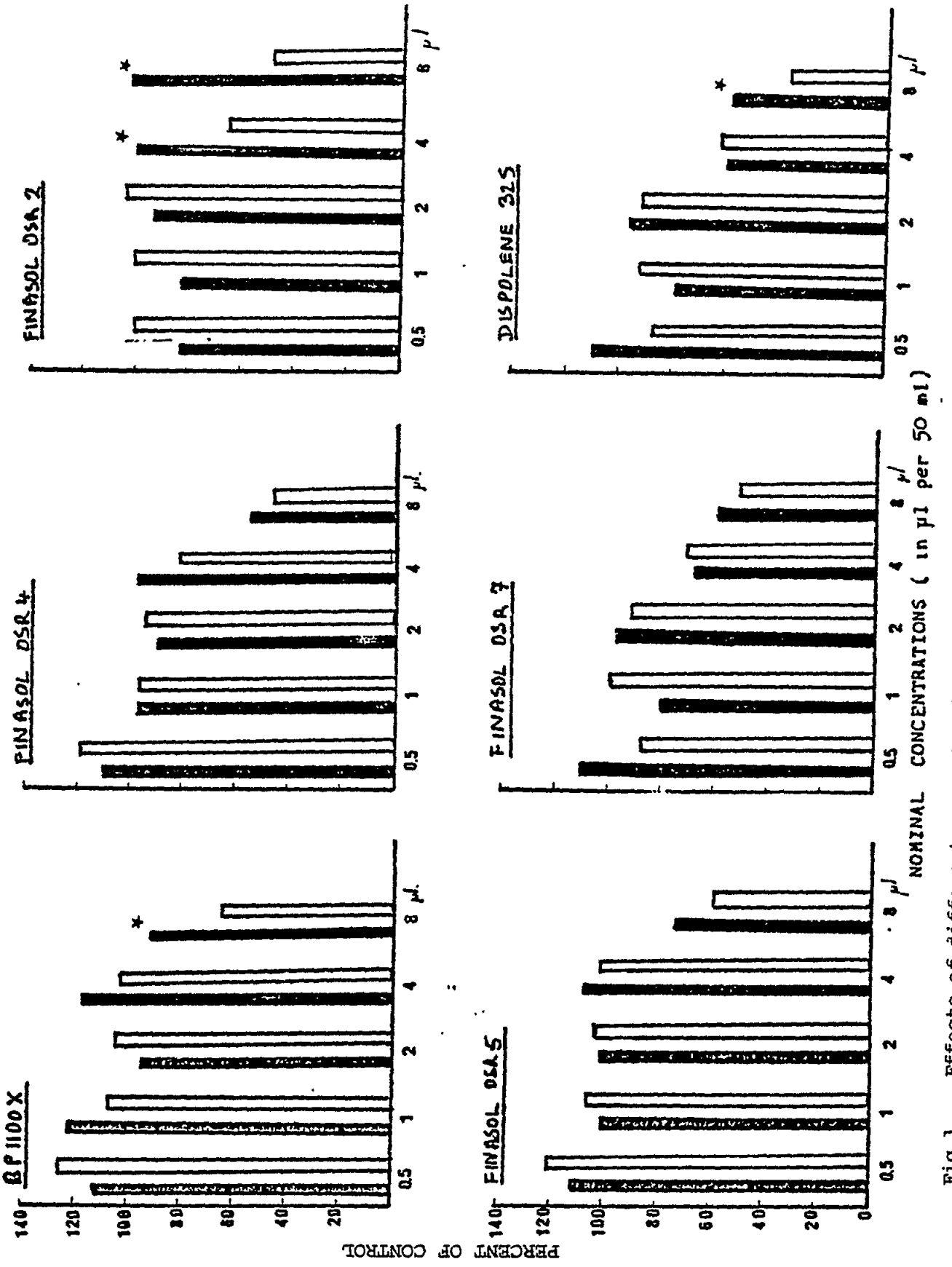

Fig.1 Effects of different concentrations of oil (black bar) or oil + dispersant mixtures (white bar) on the growth of Phaeodactylum tricornutum expressed as % of control cell counts after 24 h exposure at 20 °C, to six different dispersants. * indicates significant difference between pairs, at P = 0.05.

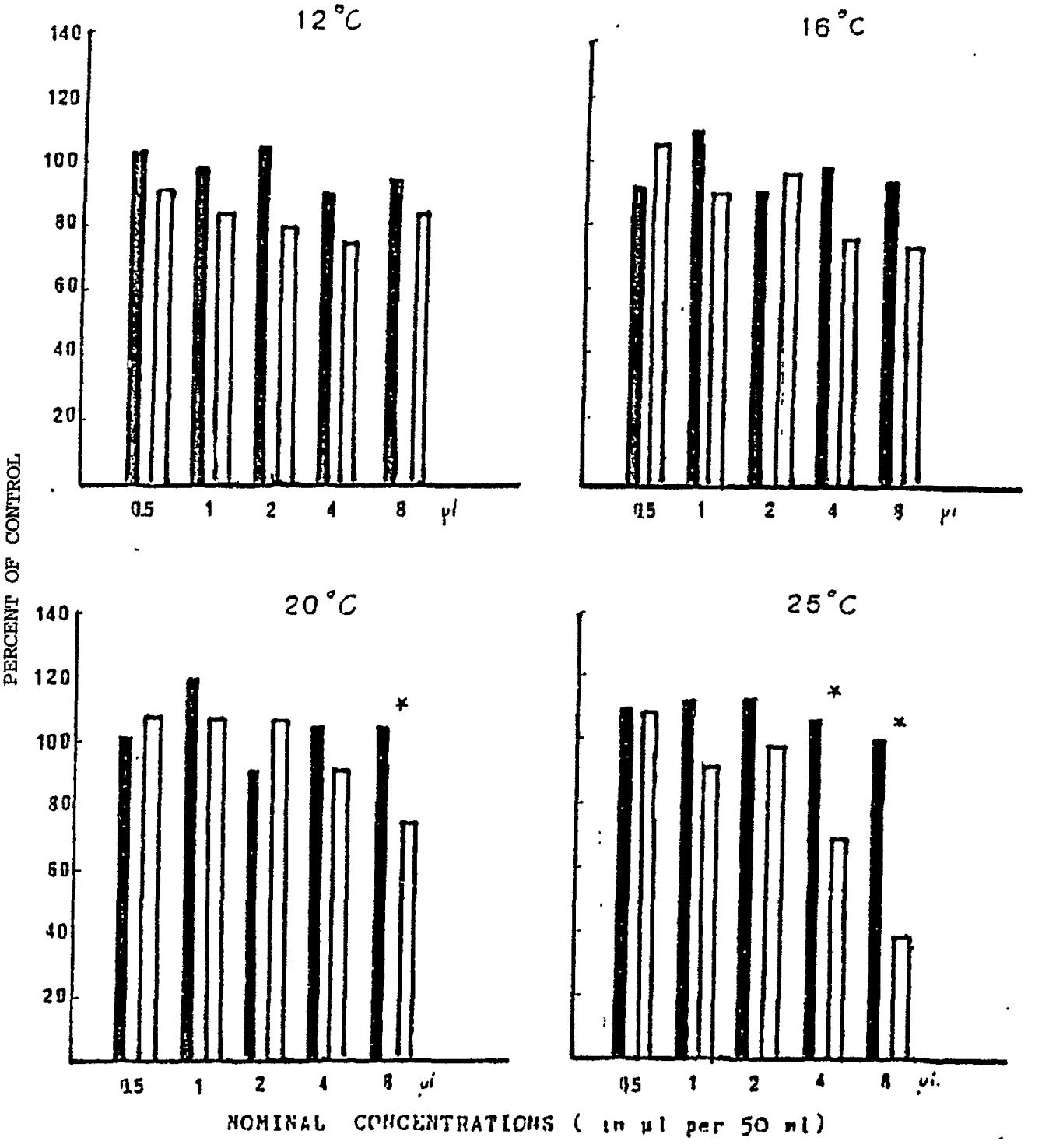

Fig.2 Effects of different concentrations of oil (black bar) or oil + BP1100X mixtures (white bar) on the growth of Phaeodactylum tricornutum, expressed as % of control cell counts after 24 h exposure at different temperatures. * indicates significant difference between pairs, at P = 0.05.

3.1.2 Conclusions

At concentrations ranging from 2 to 30 ppm, PHC chemically dispersed by any of the dispersants investigated, did not cause any toxic effect on the growth rate of this diatom which was significantly different from the effect caused by the oil alone. At higher concentrations, some of the dispersants decreased growth rates significantly as compared to controls and to growth rates on exposure to oil alone.

3.2 Patella caerulea (MOLLUSCA : GASTROPODA)

3.2.1 Toxicity of weathered crude oil and dispersants

Materials and Methods

Animals were collected from unpolluted shores and acclimated in the laboratory for 3 to 4 days. Weathered crude oil (20% loss by weight) and four dispersants (BP1100X, FINASOL OSR2, FINASOL OSR7, DISPOLENE 32S) were investigated. Treatment simulated field exposure to weathered oil on beach followed by application of dispersant. Limpets of mean shell diameter 23 mm (± 8.5 mm) were allowed to settle on perspex plates (360 x 160 mm) overnight while immersed, with 20 limpets per plate. Plates, with attached animals were then either oiled by immersing once in a tank containing surface weathered oil, so that animals acquired a light covering of oil, or treated with dispersant (applied with a hand spray gun at a rate of 0.7 cm^3 per animal) or sprayed with sea water (applied in the same manner as the dispersant), the latter serving as controls. Some of the oiled plates were either treated with dispersant (as above) after 1 hour or after 24 hours. For each run two plates per treatment, or control were used. Therefore animals were exposed either to oil, or to dispersant or to oil followed by dispersant after 1 or 24 hours. Plates were then half immersed in flowing sea water (11 cm^3 min^{-1}) in a vertical position, in separate tanks at 20 ^{o}C and 37.5 ppt. Mortality as well as position on plates were recorded after 24 and then 48 hours. Limpets failing to respond to repeated mechanical stimuli were recorded as dead.

Results

Percentage mortalities of different treatments for the four dispersants investigated are presented in Fig. 3 and Table II. No mortalities in the sea water controls were ever recorded throughout the whole investigation. Mortalities on exposure to weathered crude oil were significantly lower (Student's t-test, at $p = 0.05$) than on exposure to FINASOL OSR2 or DISPOLENE 32S alone. Highest mortalities were recorded on exposure to oil followed by dispersant after 1 hour especially for FINASOL OSR2. Applications of dispersant after 1 hour and after 24 hours of oiling did not produce significantly different mortalities.

3.2.2 Effect on respiratory activity on exposure to FINASOL OSR7

Materials and Methods

Since the toxicity experiments indicated that exposure to FINASOL OSR7 was generally not lethal to this species, it was decided to investigate any possible sublethal effects on respiration by this dispersant applied at different concentrations. Limpets of a mean shell diameter 18 mm (± 2.5 mm) were collected and acclimated as previously described. Any algal growths were

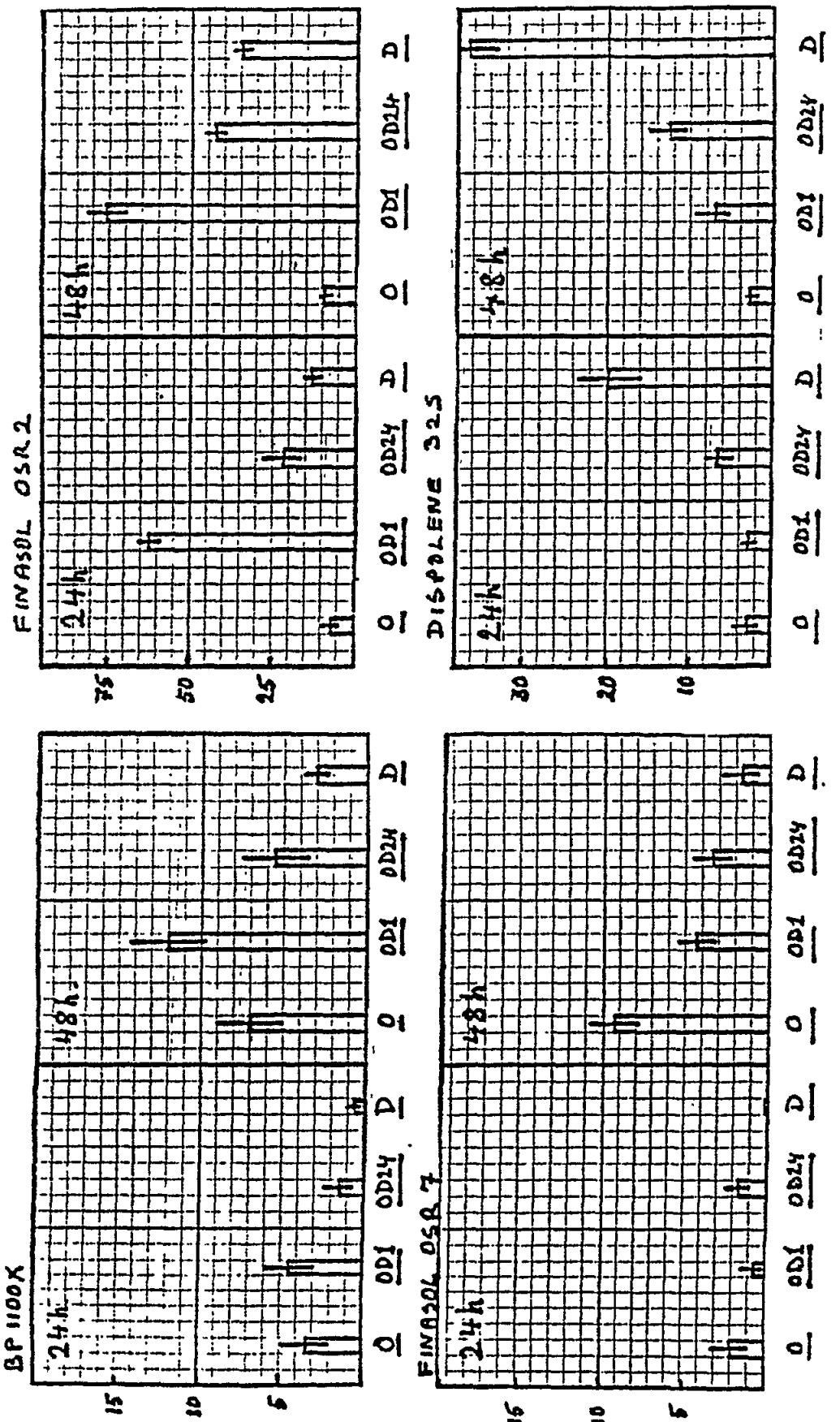

Fig.3 Patella caerulea mortality after 24 h and 48 h on exposure to:

O = weathered crude oil
OD1 = weathered crude oil followed by dispersant after 1 hour
OD24 = weathered crude oil followed by dispersant after 24 hours
D = dispersant
Means and standard deviations (number of replicates as in Table II)

Table II

Patella caerulea. Mean percentage mortality (± standard deviation) on exposure to weathered crude oil and/or dispersant. Number of replicates, with 20 animals per replicate, is indicated in brackets, in each case.

Dispersant	Time after Exposure	TREATMENTS			
		Oil	Dispersant	Oil + Disp. 1 h	Oil + Disp. 24 h
BP1100X	24h	3.5 ± 5.30(10)	0.5 ± 1.58(10)	4.5 ± 5.50(10)	1.5 ± 3.37(10)
	48h	7.0 ± 6.75(10)	3.0 ± 2.58(10)	12.0 ± 7.53(10)	5.5 ± 6.43(10)
FINASOL OSR2	24h	6.3 ± 4.79(4)	18.5 ± 4.36(4)	68.8 ± 9.47(4)	22.5 ± 10.41(4)
	48h	10.0 ± 0 (4)	35.0 ± 7.07(4)	76.3 ± 7.50(4)	43.8 ± 2.50(4)
FINASOL OSR7	24h	2.0 ± 3.47(6)	0 (6)	0.8 ± 2.04(6)	1.7 ± 2.58(6)
	48h	9.2 ± 3.76(6)	1.7 ± 2.58(6)	4.2 ± 2.04(6)	3.3 ± 4.08(6)
DISPOLENE 32S	24h	3.8 ± 4.79(4)	20.0 ± 4.08(4)	3.8 ± 4.79(4)	6.3 ± 6.29(4)
	48h	3.8 ± 4.79(4)	37.5 ± 8.66(4)	7.5 ± 5.00(4)	12.5 ± 2.89(4)

scraped off their shells. Limpets were allowed to settle on perspex plates (as above) in batches of 20 and later treated with different concentrations of dispersant (1%, 10% and 50% of FINASOL OSR7 in sea water) as indicated in the toxicity tests. Plates were allowed to stand horizontally immersed for 3 hours at 20 ^{o}C, any mortalities being recorded. Living animals still attached to the plates had their oxygen consumption monitored either immediately or after 24 hours of recovery in clean sea water. Oxygen consumption was measured by placing limpets in batches of 4, in BOD bottles of approximate volume 300 cm^3 after 3 hours. The concentration of dissolved oxygen in sea water, was determined by the Winkler titration as described by Strickland and Parsons (1972).

Oxygen consumption was then calculated by the difference between dissolved oxygen concentration in the BOD bottles with animals and that in bottles without animals (which were similarly treated). Results were expressed in ml of O_2 per hour per g of dry flesh weight.

Results

50% dilutions of FINASOL OSR7 were found to be 100% lethal while the 10% dilution resulted in 27% mortality and the 1% dilution produced 3.3% mortality. Rates of oxygen consumption after exposure to different dilutions of the dispersant are shown in Table III. A two way ANOVA of data indicated a significant effect on respiratory activity due to different treatment but not due to time. A significant F was also obtained for the interaction between time and treatment. The 1% dilution produced a slight increase in respiration which was however not significantly different to the control (Student's t test at $p = 0.05$ level) while exposure to the 10% dilution (i.e. dilution recommended by manufacturers) produced a significant decrease in respiration. Moreover, bioadhesion of limpets to perspex plates was greatly reduced on exposure to all dispersant dilutions.

Table III

Patella caerulea. Oxygen consumption rates in ml g^{-1} h^{-1} on exposure to different dilutions of FINASOL OSR7, and after 24 h recovery.

Means ± standard deviations, with number of replicates in brackets.

	TREATMENT		
	Control	1% Dispersant	10% Dispersant
Immediately after 3 h exposure	0.44 ± 0.075(10)	0.57 ± 0.094(10)	0.39 ± 0.025(8)
24 h recovery	0.47 ± 0.103(10)	0.50 ± 0.150(10)	0.53 ± 0.119(8)

3.2.3 Conclusions

These experiments (simulating exposure of this limpet to weathered crude oil on the shoreline followed by treatment with dispersants), indicate that use of FINASOL OSR2 or of DISPOLENE 32S produced more mortalities than the oil alone. Moreover while FINASOL OSR7 did not significantly increase mortalities after treatment with oil, it produced significant sublethal effects on the respiratory and bioadhesive activities of this species.

3.3 Patella rustica (MOLLUSCA : GASTROPODA)

3.3.1 Toxicity of mechanically or chemically dispersed PHC at different temperatures

Materials and Methods

Animals were collected from unpolluted shores and acclimated in the laboratory where they were allowed to settle on perspex plates for two days. Limpets were exposed to mechanically or chemically dispersed PHC in an exposure system shown in Fig. 4. The system consisted of a central Header Chamber which distributed the test emulsions at a constant rate, to 8 exposure funnels. The test emulsions were then collected in a glass chamber at the bottom from where they were pumped back to the header tank. The water temperature could be maintained at the required level with the addition of a circulating heater-chiller (Churchill Instruments) to the glass collecting chamber.

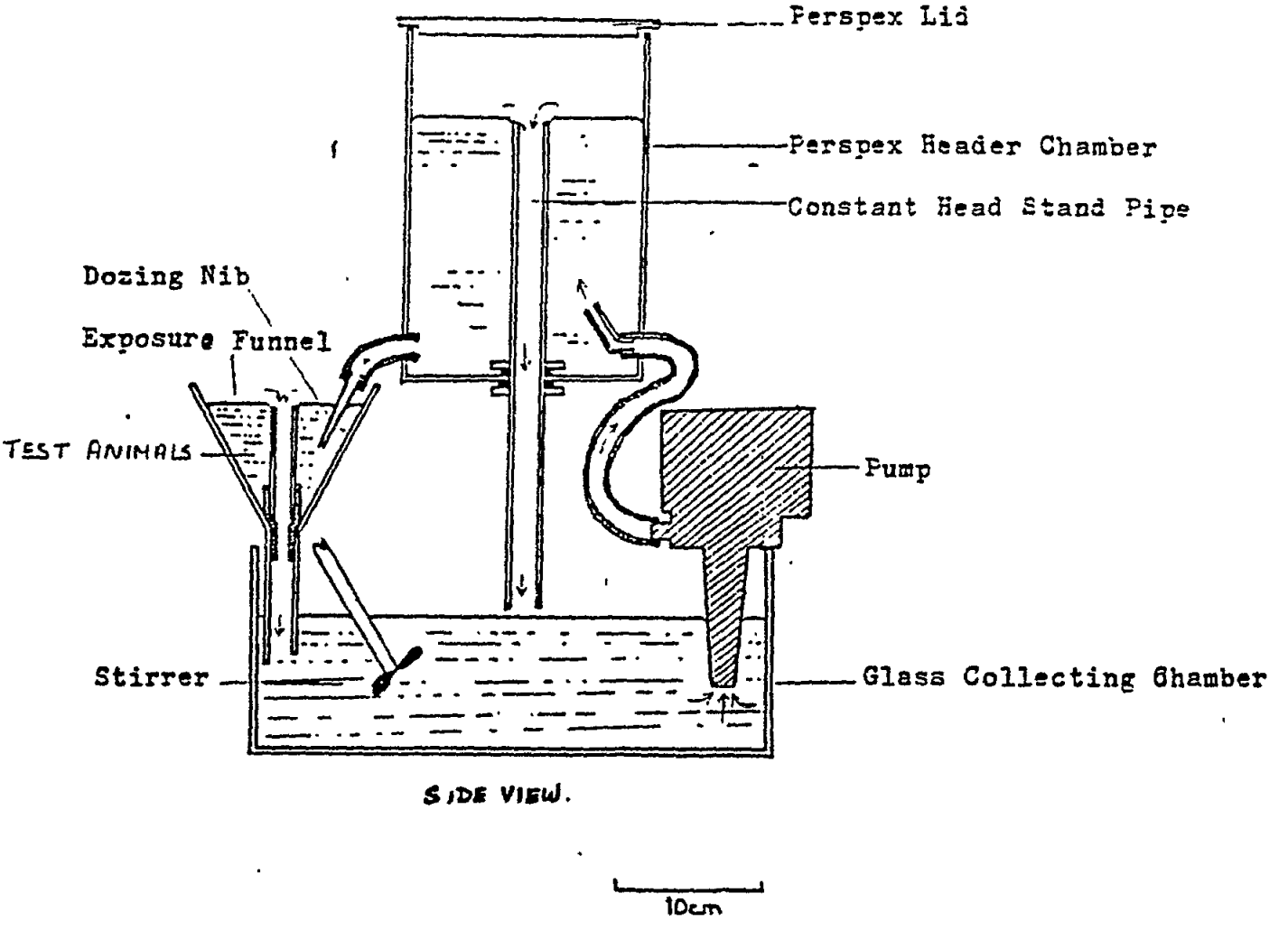

Fig.4 Exposure system for toxicity tests of oil dispersants

Five limpets were placed in each of the eight exposure funnels and the system ran for 30 minutes before exposure. Then fresh crude oil was introduced in the system at an initial nominal concentration of 0.53 cm^3 l^{-1}, and in the case of exposure to chemically dispersed oil, the same amount of BP1100X was also added. After 60 minutes of exposure, limpets were allowed to recover for 24 hours in clean aerated sea water circulated through a glass fibre filter. Mortalities after recovery were then recorded. Experiments were carried out at 14 $\pm$ 1 and 21 $\pm$ 1 ^{o}C.

Results

Investigations on the performance of the exposure system in maintaining a stable dispersion of PHC for the duration of the exposure period were carried out and results reported in previous progress reports. These results indicate that both the levels of PHC as well as the size frequency distribution of the oil droplets in the resultant dispersions were relatively constant with time over the required exposure period.

Results of toxicity tests are presented in Tables IV and V. Overall mortality in chemically dispersed oil at 14 ^{o}C is 57.5% as compared to 6.3% for mechanically dispersed oil, this difference being statistically significant (as measured by contingency X^2 comparison; X^2 = 46.05, $P < 0.001$). Overall mortality in chemically dispersed oil at 21 ^{o}C was not found to be statistically significant from that in mechanically dispersed oil at the same temperature. Contingency X^2 analysis indicated that while toxicity of mechanically dispersed PHC is independent of temperature that of chemically dispersed PHC at 14 ^{o}C was significantly higher than at 21 ^{o}C. This work is not complete yet and further experiments at higher temperatures are at present being carried out.

Table IV

Patella rustica. Mortality after 24 h recovery following 60 min exposure to oil and oil/BP1100X at a water temperature of 14 ^{o}C.

Treatment	Initial number of of limpets	Number dead after 24 h	% Mortality
Oil:			
replicate 1	40	4	10.0
replicate 2	40	1	2.5
Oil/BP1100X:			
replicate 1	40	24	60.0
replicate 2	40	22	55.0

Table VI

Patella rustica. Mean oxygen consumption (m) in ml O_2 g^{-1} h^{-1} with standard deviations (sd) and number of replicates (n), 24 h and 48 h after exposure to weathered crude oil (O); dispersant (D) or both (OD), and in sea water controls (C).

			Treatment			
Dispersant	Time		C	O	D	OD
BP1100X	24h	m	0.289	0.353	0.328	0.477
		sd	0.1525	0.2319	0.1413	0.2314
		n	10	10	10	10
	48h	m	0.471	0.598	0.385	0.409
		sd	0.3261	0.2958	0.2222	0.1262
		n	10	10	10	10
FINASOL OSR7 (10%)	24h	m	0.418	0.493	0.513	0.413
		sd	0.2750	0.1651	0.2508	0.2658
		n	10	10	10	10
	48h	m	0.516	0.305	0.318	0.442
		sd	0.2943	0.1970	0.1577	0.2539
		n	10	10	10	10
DISPOLENE 32S (1%)	24h	m	0.233	0.226	0.201	0.226
		sd	0.1003	0.0940	0.0980	0.1297
		n	8	8	8	8
	48h	m	0.201	0.210	0.134	0.155
		sd	0.0785	0.0939	0.0610	0.0897
		n	7	8	8	8

3.3.3 Conclusions

Patella rustica suffered higher mortalities when exposed to chemically dispersed oil. Moreover, preliminary studies indicate that this effect is temperature dependent. After treatment with simulating beach exposure to weathered crude oil followed by the application of dispersant, no significant effects on the respiratory activities were recorded, at the concentrations of dispersants used, except for BP1100X. However, it is to be noted that the application of DISPOLENE 32S in the recommended dilution proved to be highly lethal to this species.

3.4 Venus verrucosa (MOLLUSCA : BIVALVIA)

3.4.1 Physiological responses on exposure to chemically dispersed PHC

Materials and Methods

All animals were collected by divers from unpolluted coastal areas and acclimated in the laboratory for a minimum of three weeks. In these

Table VI

Patella rustica. Mean oxygen consumption (m) in ml O_2 g^{-1} h^{-1} with standard deviations (sd) and number of replicates (n), 24 h and 48 h after exposure to weathered crude oil (O); dispersant (D) or both (OD), and in sea water controls (C).

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3.4 Venus verrucosa (MOLLUSCA : BIVALVIA)

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Materials and Methods

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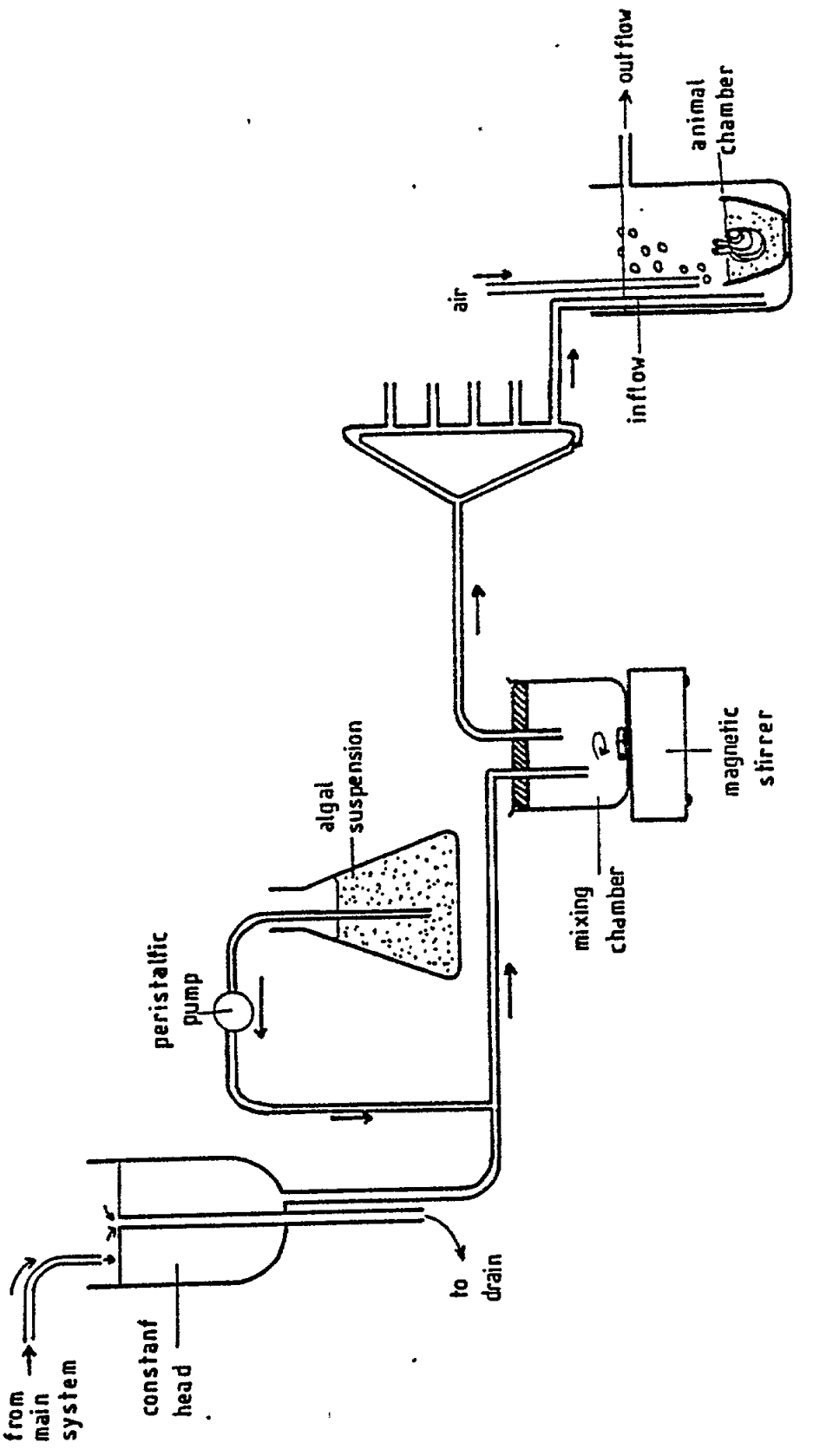

Fig. 5 Set-up for measuring clearance rates of Venus verrucosa

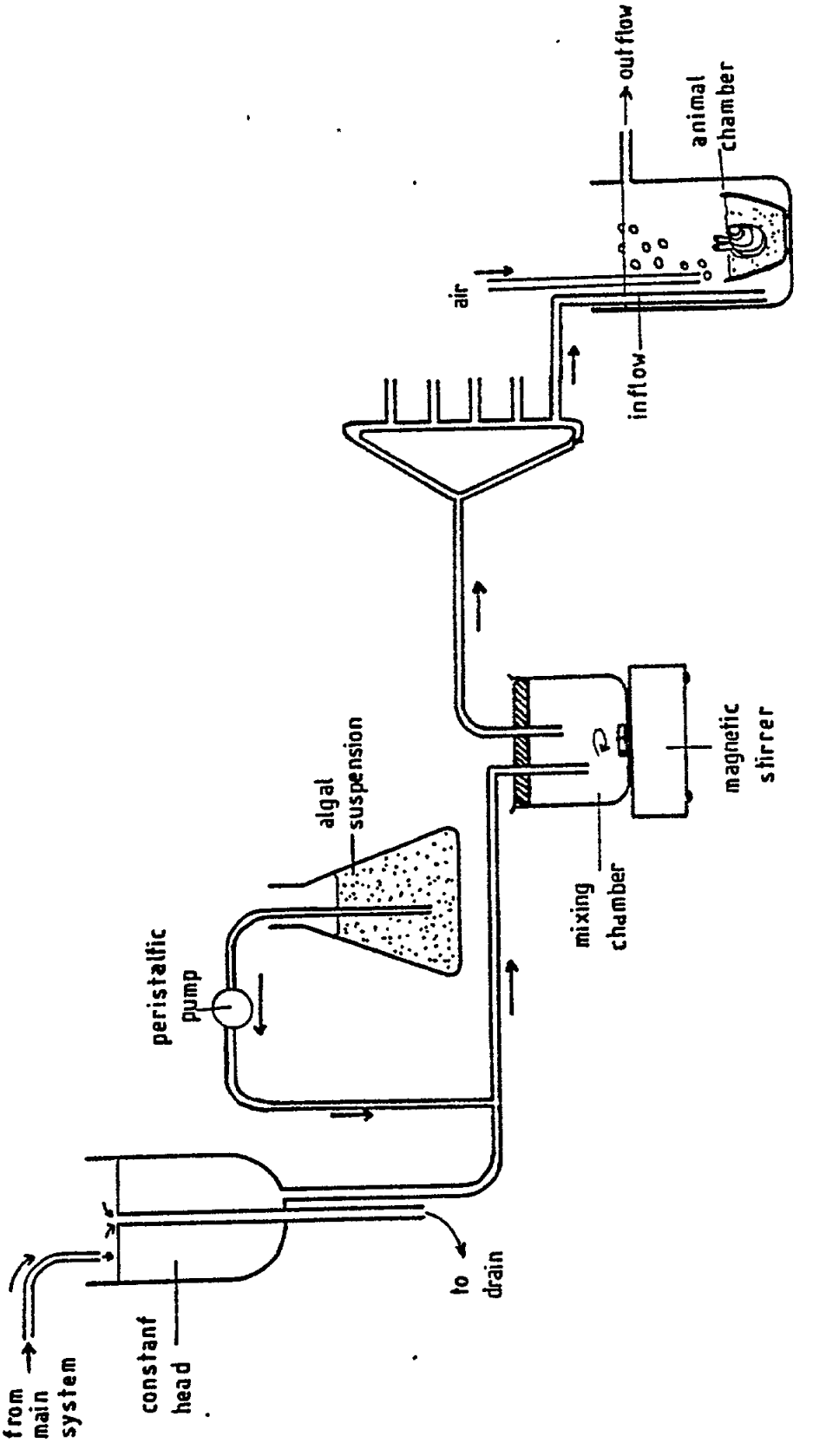

Fig. 5 Set-up for measuring clearance rates of Venus verrucosa

Second experiment

Bivalves were here exposed to a single dose of crude oil dispersed with diluted FINASOL OSR7 using the same exposure system as in the previous experiment. Physiological measurements were made on 8 animals, 5 days before dosing, and then 3, 14, 24 and 42 days after. Stress indices were calculated as previously described.

Results

First experiment

During the exposure period the levels of PHC as measured by fluorimetric spectroscopy varied from about 11 mg l^{-1} to less than 100 µg l^{-1} between successive oilings, while after 4 days, 9 µg g^{-1} dry weight of PHC in sediments and 37 µg g^{-1} weight in flesh were recorded.

Physiological responses are presented in Figs. 8.1 to 8.5, indicating a decrease in clearance rates which was statistically significant (ANOVA P = 0.05); an increase in respiration which was significantly higher than that before exposure and a significant increase in ammonia excretion at 4, 11 and 32 days after exposure. The assimilation or food absorption efficiency declined from 83% to 70% after 4 d exposure and was subsequently recorded as 72% and 75% by the 11 d and 32 d respectively.

At day 4, there was a significant decrease in SFG followed by a recovery after 32 days, and significant decrease in O:N ratio which persisted after 32 days.

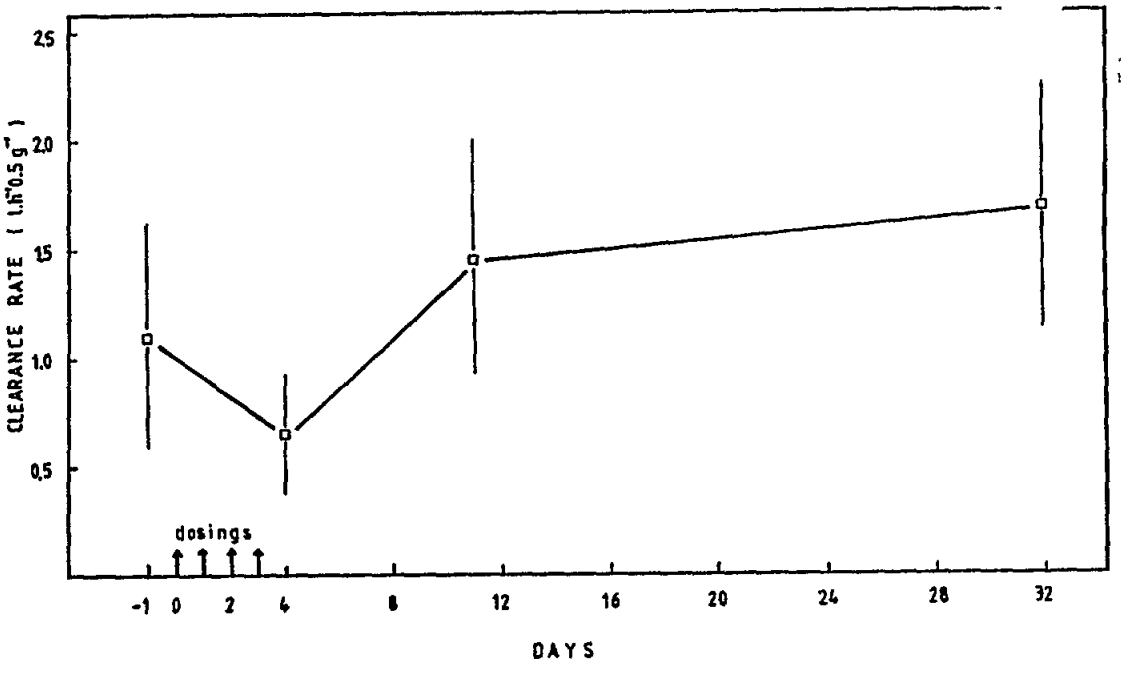

Fig. 8.1 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Clearance rates (Means and 95% confidence limits) - Experiment 1

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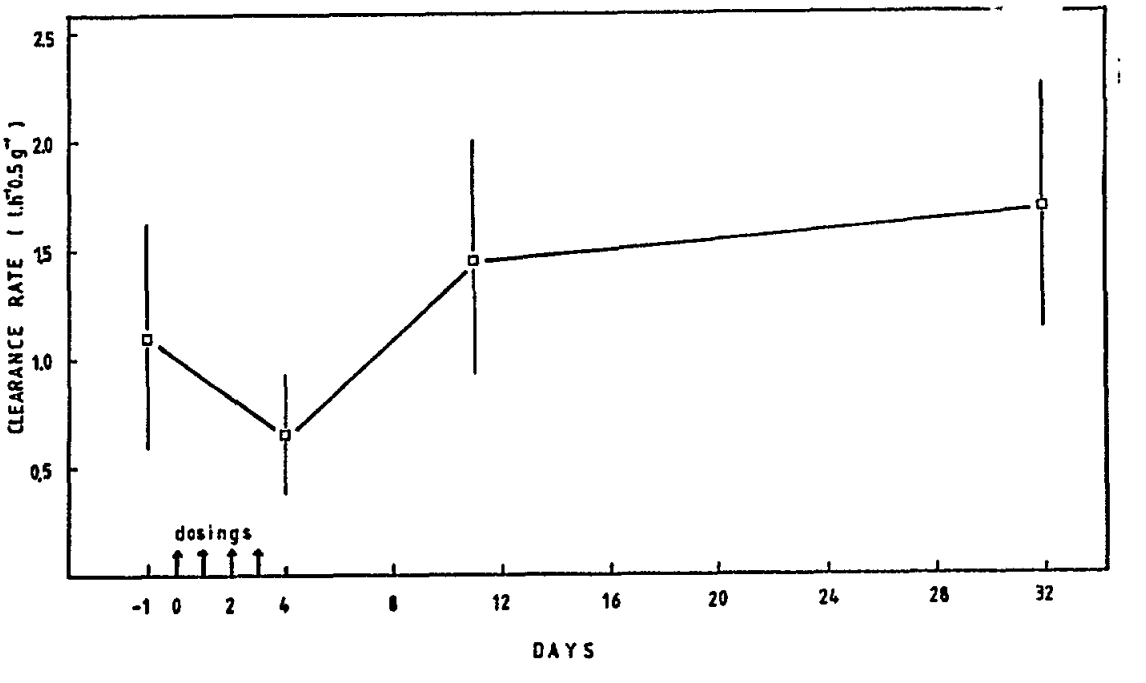

Fig. 8.1 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Clearance rates (Means and 95% confidence limits) - Experiment 1

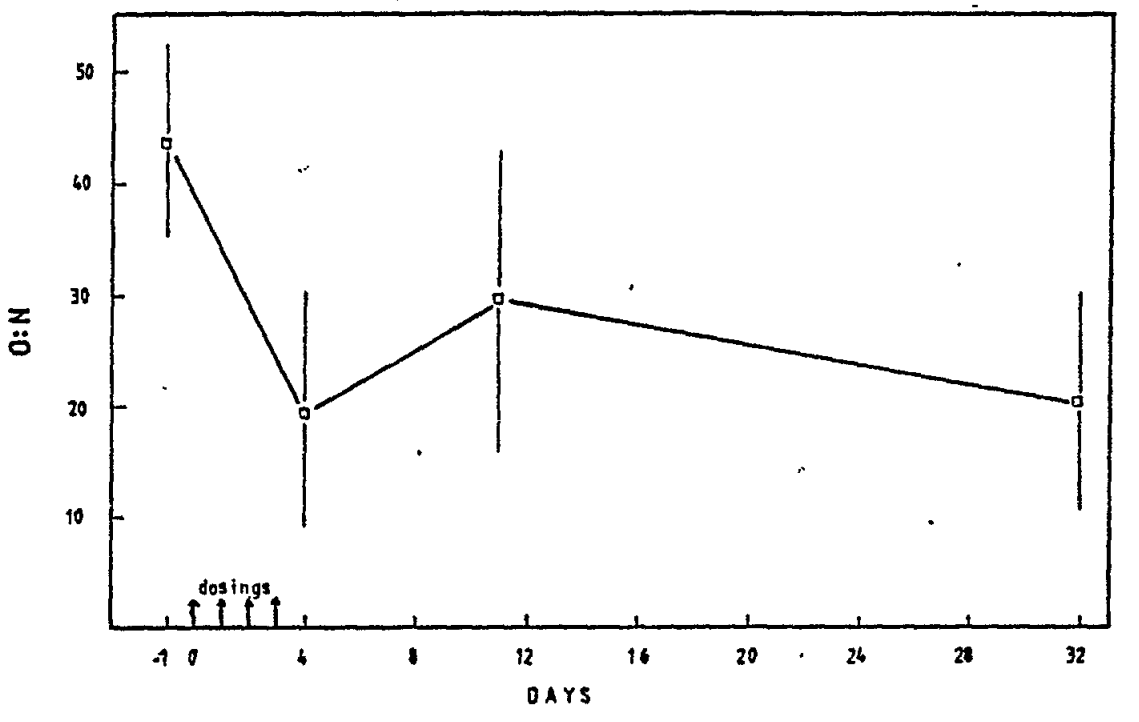

Fig. 8.4 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Oxygen to Nitrogen ratio (Means and 95% confidence limits) - Experiment 1

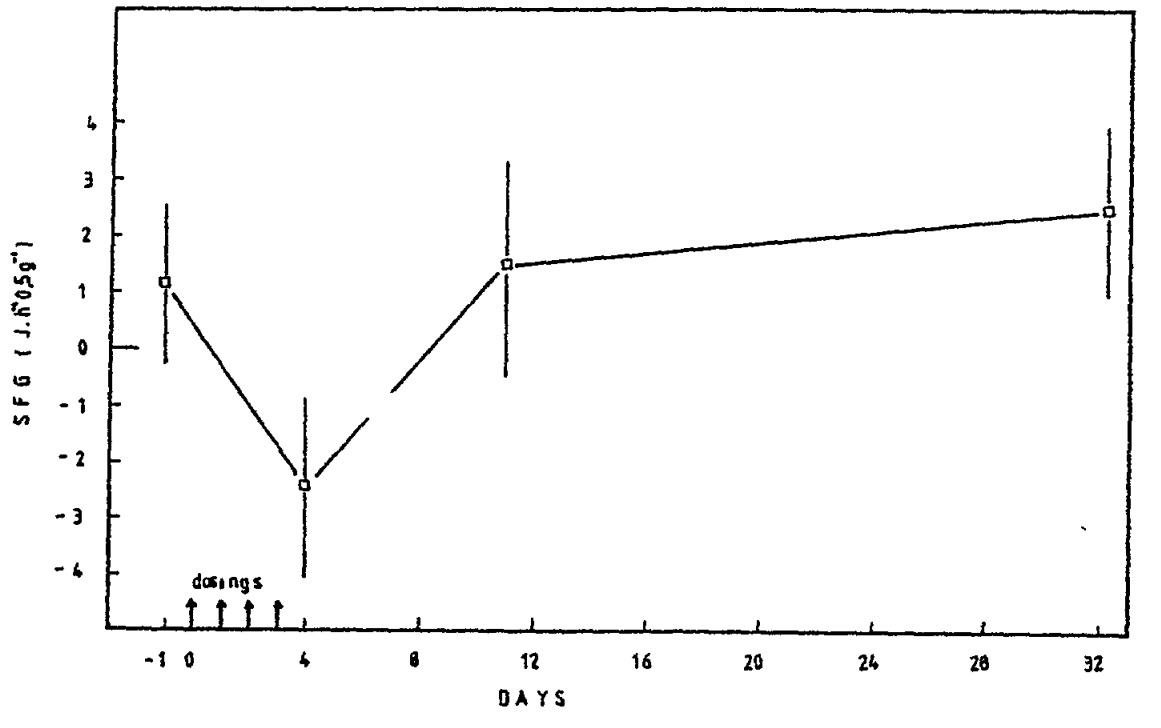

Fig. 8.5 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Scope for growth (Means and 95% confidence limits) - Experiment 1

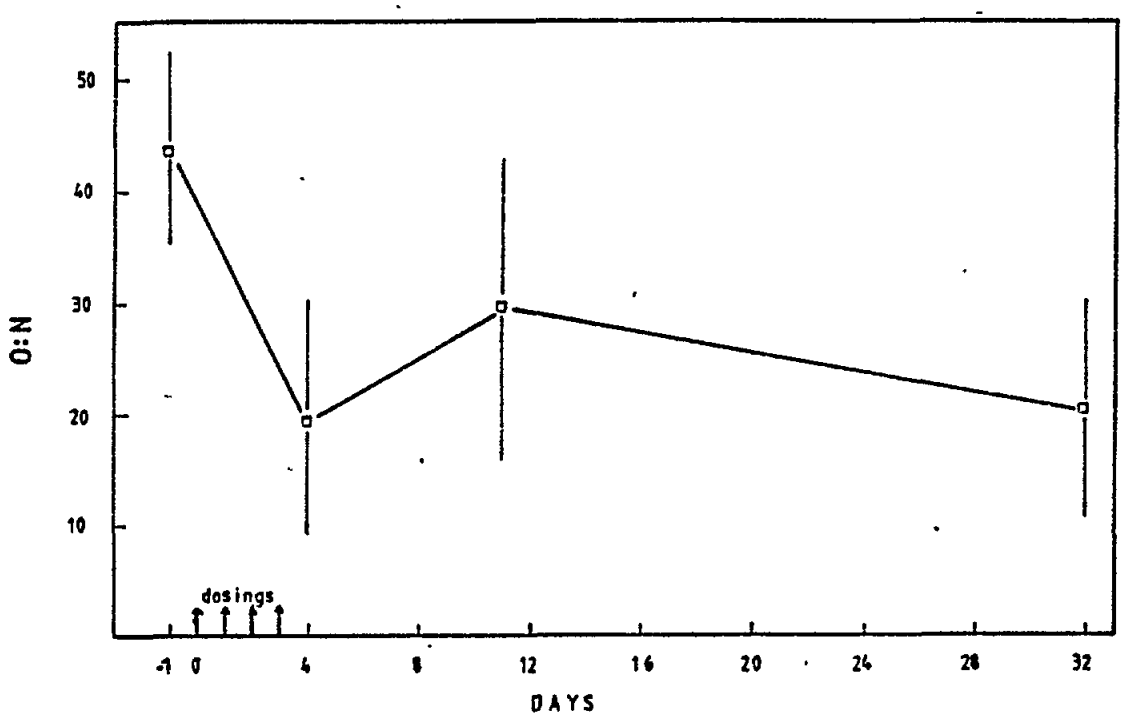

Fig. 8.4 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Oxygen to Nitrogen ratio (Means and 95% confidence limits) - Experiment 1

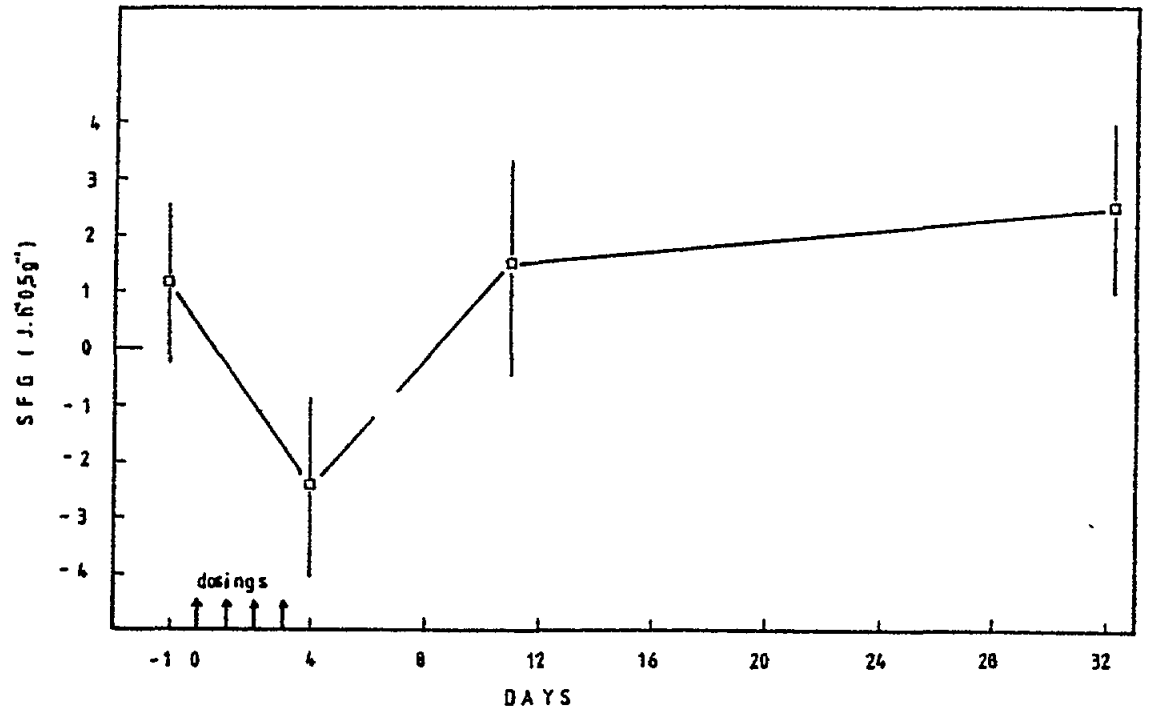

Fig. 8.5 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Scope for growth (Means and 95% confidence limits) - Experiment 1

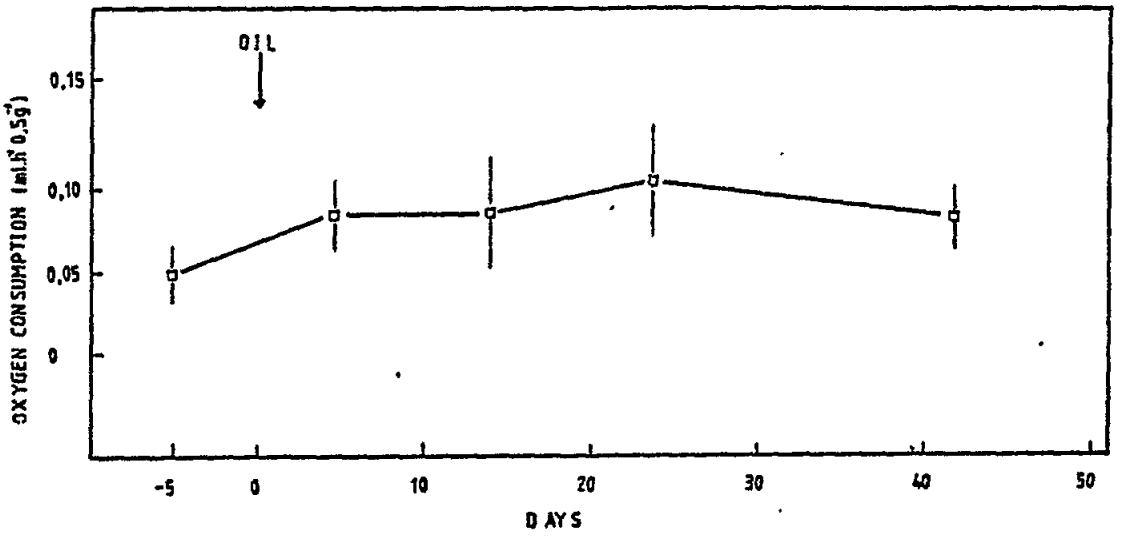

Fig. 9.2 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Oxygen consumption (Means and 95% confidence limits) - Experiment 2

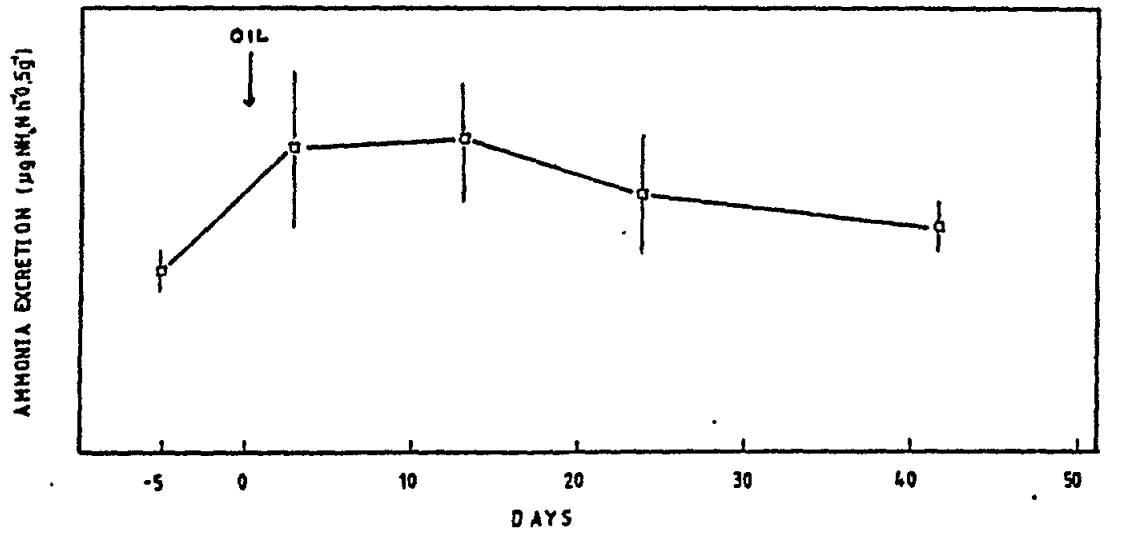

Fig. 9.3 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Ammonia excretion (Means and 95% confidence limits) - Experiment 2

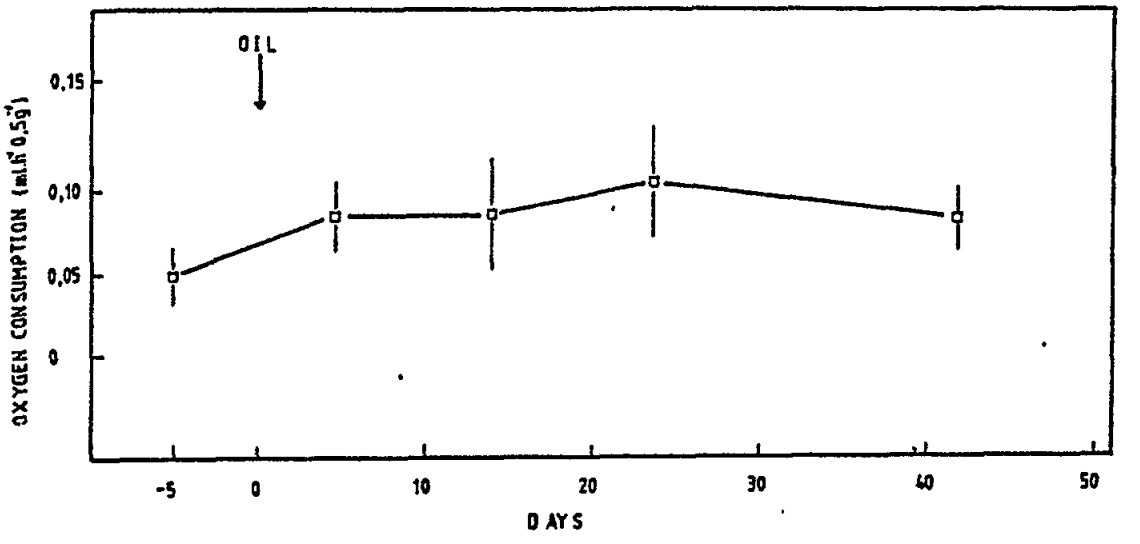

Fig. 9.2 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Oxygen consumption (Means and 95% confidence limits) - Experiment 2

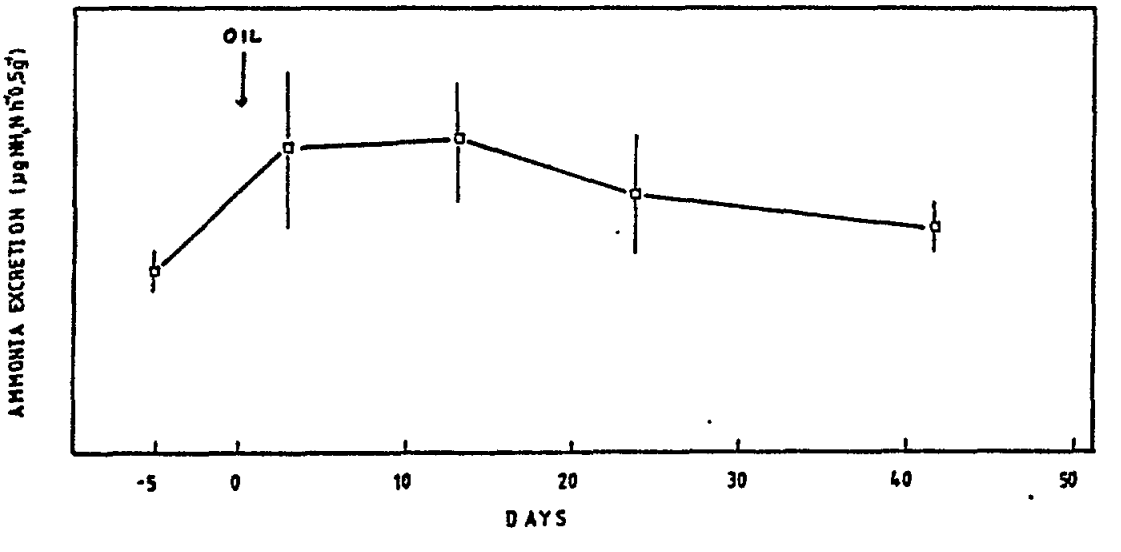

Fig. 9.3 Venus verrucosa. Physiological responses on exposure to chemically dispersed petroleum hydrocarbons. Ammonia excretion (Means and 95% confidence limits) - Experiment 2

3.5 Paracentrotus lividus (ECHINODERMATA : ECHINOIDEA)

3.5.1 Effects on the survival and spinal/tube feet responses on exposure to mechanically or chemically dispersed crude oil

Materials and Methods

Animals were collected by divers from unpolluted localities on the NE coast of Malta and acclimated in the laboratory for at least three days before use. Sea urchins were exposed in batches of 5 in plastic tanks containing 5 litres of test solutions. Oil dipersion was prepared by vigorously agitating varying amounts (0.1, 0.5, 1 and 5 cm^3) of oil plus dispersant (equal volumes of 0.1, 0.5, 1 and 5 cm^3) in 1 litre of sea water, in a 2.5 litre volume bottle. The resultant emulsions were then added to the experimental tanks and volumes made up to 5 litres. Test mixtures were left unaerated during the exposure period to minimize losses in PHC levels. Mortalities after 24 hours and 48 hours were then recorded. Animals which failed to respond to repeated mechanical stimuli applied on the test, and which lost all bioadhesion of tube feet to the aquaria walls, were recorded dead. Spinal and tube feet response to localized mechanical stimuli applied on test with a sharp metallic needle, after 24 hours and 48 hours exposure were recorded and scored as indicated in Table VIII. Two dispersants were investigated (BP1100X and FINASOL OSR7).

Results

Data on mortality are presented in Table VII. Effect by mechanically dispersed oil was dependent on concentrations as well as exposure time. At any particular nominal concentration, chemically dispersed mixtures were more lethal than mechanically dispersed mixtures. (The difference being significant at the $P = 0.05$ level for most cases, as analysed with Student's t-test). Changes in actual levels of PHC were monitored during the experiment. For example, in the case of 0.5 cm^3 of oil per 5 l of sea water, from an initial concentration of 9.5 ppm, levels decreased to 2.9 ppm after 24 hours and to 1.93 ppm after 48 hours. In the case of 0.5 cm^3 of oil and 0.5 cm^3 of dispersant per 5 l of sea water, an initial concentration of 26.8 ppm decreased to 8.5 ppm after 24 hours and to 6.5 ppm after 48 hours.

Results on effects on spinal/tube feet responses are presented in Table VIII. Such behavioural responses of spines and tube feet became sluggish or greatly reduced especially on exposure to the chemically dispersed PHC. These investigations have not been complete yet. Further data are expected, before non-parametric statistical tests of significance are applied.

3.5.2 Effects of oil and oil dispersants on respiratory activity

Material and Methods

Animals were collected from unpolluted localities on the NE coast of Malta, and were acclimatized for at least three days but were used within 10 days of collection.

Selected healthy animals were transferred to glass respirometers (Section 3.4.1) supplied from the same sea water circulating system as the holding tanks and allowed to adapt to these overnight. To measure oxygen consumption the respirometers were shut off from the sea water supply and the urchins allowed to deplete the oxygen in the ambient water. The water in the respirometers was kept stirred by means of a bar magnet and magnetic stirrer arrangement.

Table VII

Paracentrotus lividus. Mean percentage mortality (m), together with standard deviations (sd) and number of replicates (n) on exposure to oil + dispersant mixtures.

Dispersant	Exposure		Treatment+ C	$O_{0.1}$	$O_{0.5}$	O_5	$OD_{0.1}$	$OD_{0.5}$	OD_1	OD_5
BP1100X	24h	m	0	0	2	56.7	8	22.5	14	100
		sd	0	0	6.32	26.58	13.98	27.4	16.32	0
		n	10	10	10	6	10	10	6	6
	48h	m	0	0	10	76.6	22.5	70	63.3	100
		sd	0	0	10.7	23.38	19.82	23.90	19.66	0
		n	8	8	8	6	8	8	6	6
FINASOL OSR7	24h	m	0	2.9	2.9	20	11.4	22.9	24	100
		sd	0	10.69	10.69	23.09	23.16	35.00	27.97	0
		n	14	14	14	10	14	14	10	10
	48h	m	0	8.3	8.3	-	11.7	35	32	100
		sd	0	23.29	19.92	-	30.10	37.29	41.31	0
		n	12	12	12	-	12	12	10	10

+ C = sea water control; $O_{0.1}$ = 0.1 ml oil/5 l; $O_{0.5}$ = 0.5ml oil/5 l; O_5 = 5 ml oil/5 l; $OD_{0.1}$ = 0.1 ml oil + 0.1 ml dispersant/5 l; $OD_{0.5}$ = 0.5 ml oil + 0.5 ml dispersant/5 l; OD_1 = 1 ml oil + 1 ml dispersant/5 l; OD_5 = 5 ml oil + 5 ml dispersant/5 l of test solutions.

Table VIII

Paracentrotus lividus. Effect on spinal and tube feet response to localized mechanical stimuli on exposure to oil or oil + oil dispersant mixtures.

Response score: 1 - immediate local and general spinal response
2 - slow local and general spinal response
3 - slow local spinal response only
4 - no local spinal response except for random movement
5 - no response

Data presented as mean response score ($\bar{r}$); standard deviation (sd) and number of respicates (n) after 24 h and 48 h exposure to 0.1 ml ($O_{0.1}$); 0.5 ml ($O_{0.5}$); per 5 l of test solutions or 0.1 ml of oil + 0.1 of dispersant ($OD_{0.1}$); 0.5 ml of oil + 0.5 ml of dispersant ($OD_{0.5}$) per 5 l of test solutions.

			Treatment				
Dispersant	Exposure		C	$O_{0.1}$	$O_{0.5}$	$OD_{0.1}$	$OD_{0.5}$
BP1100X	24h	$\bar{r}$	1.7	2.0	2.9	3.3	4.4
		sd	0.89	0.78	0.84	0.66	1.70
		n	50	50	49	46	41
	48h	$\bar{r}$	2.3	4.2	3.3	4.2	4
		sd	1.24	4.31	1.00	1.76	0.95
		n	40	44	38	30	12
FINASOL OSR7	24h	$\bar{r}$	1.4	1.5	2.1	2.0	3.3
		sd	0.51	0.63	0.93	1.07	1.16
		n	70	68	68	62	53
	48h	$\bar{r}$	2.0	1.8	2.2	3.3	3.8
		sd	1.34	1.21	1.21	6.01	0.97
		n	68	61	57	50	31

At intervals, samples of between 0.5 and 1.0 cm^3 of respirometer water were withdrawn by means of a syringe, at the same time introducing an equivalent amount of water as that removed from the compensating syringe. The oxygen content of the sampled water was then measured with an Instrumentation Laborary 213 pH Blood Gas Analyser fitted with an Instrumentation Laboratory 227 Temperature Controller.

The urchins were then removed from the respirometers and placed in the exposure apparatus shown in Fig. 10. Crude oil was added to the mixing chamber of the exposure apparatus to give an overall nominal initial concentration of oil of 0.01 cm^3 l^{-1}. For those experiments on oil/dispersant mixtures, the apparatus was dosed with oil as above and within one minute of dosing, dispersant was introduced into the mixing chamber to

give an overall nominal initial concentration of dispersant of 0.01 cm^3 l^{-1}. Dispersants tested were BP1100X applied neat and DISPOLENE 32S diluted 1:9 with sea water before application as per the manufacturer's instructions. Urchins were left in the exposure apparatus for 20 - 24 hours and then removed, rinsed in clean sea water and introduced into the respirometers and allowed to recover for 1 hour. Respiration was then measured as described previously. In some experiments respiration after 24 hours recovery in the respirometers was also measured. Finally the urchins were removed, rinsed in distilled water, dried in an oven at 90^{oC} to constant weight and weighed.

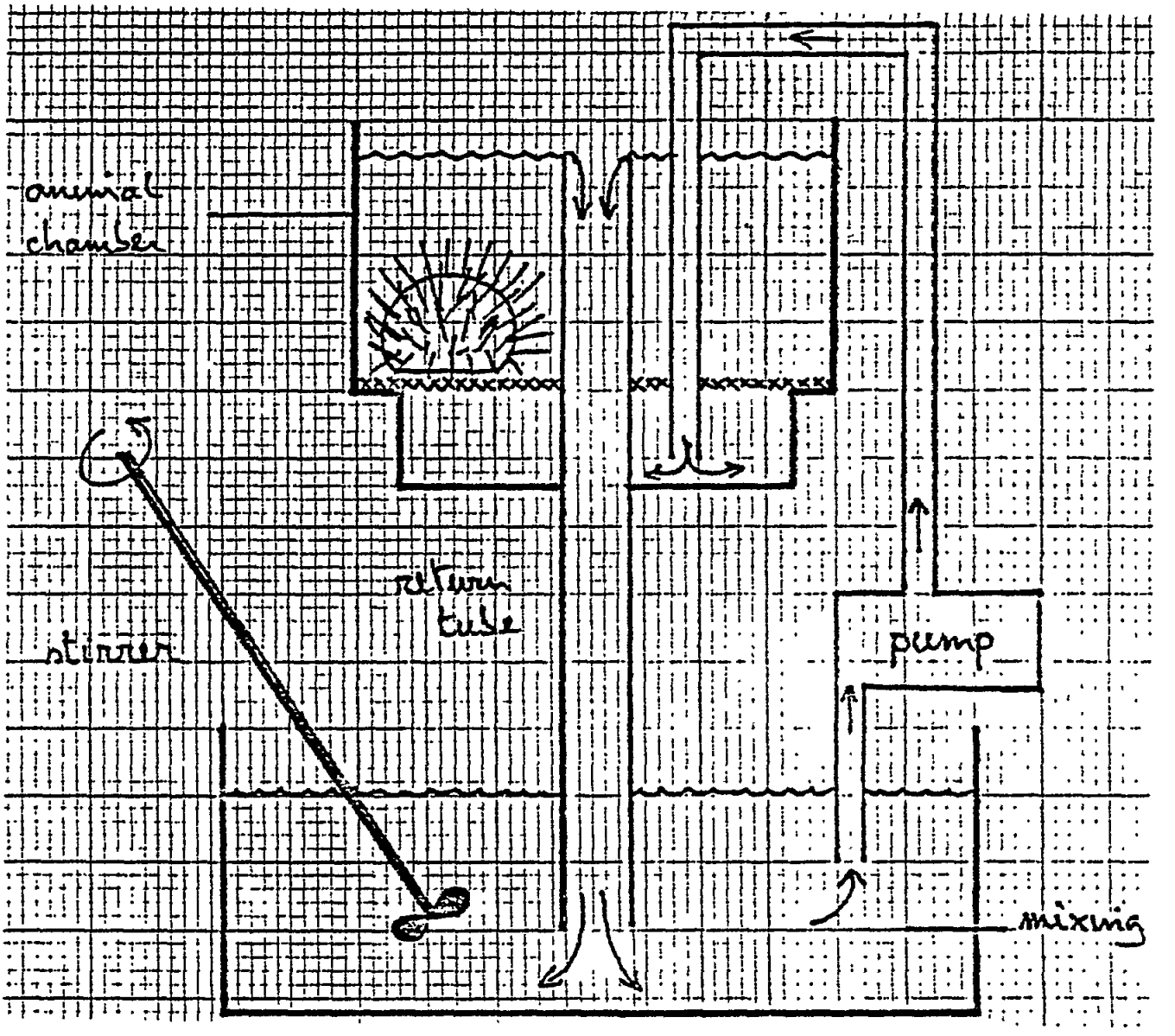

Fig.10 Paracentrotus lividus. Exposure system (Section 3.5.2)

Respiration measurements and exposure to oil and oil/dispersant mixtures were carried out at a water temperature of 20 $^{o}C \pm 1$ and salinity of 37 ppt $\pm$ 1.5 under a luminous intensity of 120 lux.

Results

Regulation of oxygen consumption

To measure regulatory ability, four urchins were allowed to deplete the oxygen in water from ambient tensions (c.150 mm Hg) to c.80 mm Hg. A plot of weight specific oxygen consumption (VO_2) against oxygen tension (pO_2) showed no trend indicating that over the tested range of pO_2, *P. lividus* behaves as an oxyconformer. To further test this, the plots of VO_2 against pO_2 were transformed to linear form using the method of Tang (1933) by plotting pO_2/VO_2 against pO_2 and calculating the ratio K_1/K_2 (where K_1 = intercept and K_2 = slope of the Tang plot) (Bayne, 1971). A low value of K_1/K_2 indicates a greater capacity to regulate oxygen consumption than a high value. The individual values obtained are reported in Table IX.

Individually and combined ($x = 64.53 \pm 28.36$ s.d.) these values are quite low confirming that VO_2 is independent of ambient pO_2 over the range of oxygen tensions tested.

Table IX

Paracentrotus lividus. Values of the ratio K_1/K_2 obtained from plots of Po_2/Vo_2 against po_2 for four sea urchins where K_1=intercept and K_2=slope of the calculated regression lines for these plots.

Urchin No.	Dry weight (g)	K_1/K_2
1	19.74	-77.26
2	14.53	-87.21
3	14.59	-70.39
4	20.00	-23.28

Oxygen consumption and body weight

The relationship between whole animal oxygen consumption and body weight (W) is described by the equation:

$$\text{oxygen consumption} = aW^b.$$

The relationship between weight specific oxygen consumption (VO_2) and body weight is therefore:

$$VO_2 = aW^{b-1}$$

or adopting the notation of Davies (1966):

$$V02 = aW^b$$

where $b' = b - 1$

Taking logs: $\log VO_2 = \log a + b' \log W$

In the present investigation the oxygen consumption of a total of 52 urchins ranging in dry body weight from 3.61 to 20.16 g was measured under control conditions (i.e. not exposed to oil or oil/dispersant). Using these data, body weight was found to be highly correlated with weight specific oxygen consumption (Pearson product moment correlation r = -0.434, d.f. = 50, $0.001 < P < 0.002$). The regression equation describing this relationship is:

$$\log VO_2 = -1.189 - 0.521 \log W$$

giving values for a and b' of 0.0648 and -0.521 respectively.

Regression equations for the plots of log VO_2 and log W were also calculated separately for each of the three treatments tested (oil alone, oil/BP1100X and oil/DISPOLENE 32S) and their controls. These results are given below:

<u>treatment = oil</u>

control: $\log VO_2 = -1.083 - 0.556 \log W$
($r = -0.454$, d.f. = 18, $0.02 P < 0.05$*)

1 h recovery: $\log VO_2 = -1.234 - 0.451 \log W$
($r = -0.596$, d.f. = 14, $0.01 < P < 0.02$*)

24h recovery: $\log VO_2 = -1.445 - 0.212 \log W$
($r = -0.523$, d.f. = 6, $P < 0.50$ NS)

<u>treatment = oil/BP1100X</u>

control: $\log VO_2 = -1.197 - 0.519 \log W$
($r = 0.523$, d.f. = 18, $0.01 < P < 0.02$*)

1 h recovery: $\log VO_2 = -1.227 - 0.459 \log W$
($r = -0.724$, d.f. = 14, $0.001 < P < 0.002$**)

24h recovery: $\log VO_2 = -0.060 - 1.478 \log W$
($r = -0.954$, d.f. = 6, $P < 0.001$***)

<u>treatment = oil/DISPOLENE 32S</u>

control: $\log VO_2 = -1.058 - 0.729 \log W$
($r = -0.352$, d.f. = 10, $0.20 < P < 0.50$ NS)

1 h recovery: $\log VO_2 = -0.602 - 0.994 \log W$
($r = 0.613$, d.f. = 10, $0.02 < P < 0.05$*)

Note: * = significant at P = 0.05
** = significant at P = 0.01
*** = significant at P = 0.001
NS = not significant

These regression equations were compared statistically for differences in slope using Student's t. There is no significant difference (at P = 0.05) between the slopes of the control and 1 h recovery, and control and 24 h recovery equations for the oil experiment. Similarly for the oil/BP1100X experiment. No significant difference was found (at P = 0.05) between the

slopes of the control and 1 hr recovery equations for the oil/DISPOLENE 32S experiment. It appears therefore that neither oil alone nor oil mixed with the two dispersants tested has any effect on the relationship between body weight and weight specific oxygen consumption under the experimental conditions employed in this study.

Effect of oil and oil/dispersant on VO_2

Levels of PHC in the test media as monitored over the 24 h exposure period are presented in Table X.

Table X

Paracentrotus lividus. Levels of PHC during exposure period in $\mu g\ l^{-1}$. (Section 3.5.2.2).

Time after dosing	Oil	Oil+BP1100X	Oil+DISPOLENE 32S
30 min	2233	2685	1102
8 h	33	1159	1200
24 h	154	270	3310

The values of VO_2 obtained for the urchins before treatment with oil/dispersant and after 1 h recovery following treatment were compared statistically using the paired-sample t-test (Zar, 1974). The results of this analysis are shown in Table XI.

Table XI

Paracentrotus lividus Respiration. Results of paired-sample t-test analysis of VO_2 values before treatment and following 1 h recovery after exposure to oil or oil/dispersant mixtures.

Treatment	t.s.	d.f.	significance
oil	0.782	15	NS
oil/BP1100X	1.222	15	NS
oil/DISPOLENE 32S	2.295	11	+

+ = significant at P=0.05; NS=not significant

There is no significant difference between weight specific oxygen consumption before and after exposure to oil following a recovery period of 1 h (VO_2 before exposure: $\bar{x}$ = 0.0201 $cm^3\ O_2\ g^{-1}\ h^{-1}$ ± 0.0050 s.d.; after exposure: $\bar{x}$ = 0.0183 $cm^3\ O_2\ g^{-1}\ h^{-1}$ ± 0.0034 s.d.); similarly

for exposure to oil/BP1100X mixtures (VO_2 before exposure; $\bar{x}$ = 0.0226 cm^3 O_2 g^{-1} h^{-1} $\pm$ 0.0112 s.d; after exposure: $\bar{x}$ = 0.0285 cm^3 O_2 g^{-1} $\pm$ 0.0091 s.d.). However following exposure to oil/DISPOLENE 32S, weight specific oxygen consumption increased by 32.4% (VO_2 before exposure: $\bar{x}$ = 0.0165 cm^3 O_2 g^{-1} h^{-1} $\pm$ 0.0103 s.d.; after exposure: $\bar{x}$ = 0.0219 cm^3 O_2 g^{-1} h^{-1} $\pm$ 0.0083 s.d.).

A similar analysis was performed for the oil/BP1100X experiment testing differences in VO_2 before exposure and after 24 h recovery following exposure. In both cases, and as expected there was no significant difference in weight specific oxygen consumption (at p = 0.05) (VO_2 following 24h recovery; oil $\bar{x}$ = 0.0222 cm^3 O_2 g^{-1} h^{-1} $\pm$ 0.0081 s.d.); oil/BP1100X: $\bar{x}$ = 0.0247 cm^3 O_2 g^{-1} h^{-1} $\pm$ 0.0099 s.d.).

3.5.3 The effects of oil and oil dispersants on locomotory behaviour

Materials and Methods

Animals were collected from unpolluted localities on the NE coast of Malta, transferred to the laboratory, and acclimatized to laboratory conditions. Animals were used for experiments after a three day acclimatization period but within 10 days of collection.

All experiments were carried out in a dark room where the only illumination was provided by a small 15 W red bulb giving a luminous intensity of 25 lux at the surface of the experimental aquaria. During all experiments the water temperature was maintained at 20 $^{o}C \pm 1$ and salinity at 37 ppt$\pm$1.5.

Righting behaviour

Experiments on righting behaviour were carried out in a large aquarium (42 x 1.6 x 20 cm) filled with clean sea water from the same supply as that feeding the holding tanks. To time righting, a sea urchin was taken out of the water and immediately placed aboral surface down in the centre of the experimental aquarium and timing started. Timing was stopped when the mouth region of the urchin again made contact with the bottom. Three times were recorded: latency time, defined as that period between first contact with the bottom and first movement of the urchin (usually in the form of a "jerk" as the spines lever the animal up); righting time, defined as that period between first movement and first contact of the mouth region with the bottom; total righting time, defined as the sum of latency time and righting time.

The great majority of animals righted completely in less than 5 minutes. Those animals which took longer than 6 minutes to right were considered unhealthy and discarded. Similarly, if the urchin made contact with the sidewalls of the aquarium at any time during the righting sequence, that result was discarded.

Each urchin was subjected to three righting trials and the average of times recorded taken as the values for that urchin. Urchins were then exposed for 24 hours to either fresh oil or oil and dispersant mixtures (BP1100X) in the same exposure apparatus using the same concentrations of oil and dispersant as already described.

Following exposure to oil or oil/dispersant, the urchins were removed from the exposure apparatus, rinsed in clean sea water and righting measured as described above (three trials per urchin). The urchin was then rinsed in distilled water, dried in an oven at 90 ^{o}C to constant weight and weighed. The spines were then brushed off the test and maximum test diameter, maximum test height and diameter of the peristome opening measured with vernier calipers.

Movement

For these experiments an identical aquarium to that used for righting experiments was used except that this had the bottom marked out in a grid of squares each of side 6 cm. An urchin was placed oral surface down in the central square of the grid and timing started. Every two minutes its position on the grid was recorded, taking the axis running through the urchin's mouth and anus as the reference point for its position. The experiment was stopped when the urchin remained in the same grid square for five consecutive 2 min. periods. Usually urchins stopped moving when they reached the corners of the aquarium; less frequently they stopped moving on reaching the sides. Maps of the paths taken were drawn up and from these total distance moved (calculated as the linear distance between one position record and the next), speed of movement (calculated from total distance moved and total time taken to complete this distance) and rate of turning (calculated from number of changes of direction from linear movement during the journey and time taken to complete journey) for each urchin were determined. Three trials per urchin were made and the average speed and turning rate recorded during these taken as the values for that particular urchin.

Urchins were then exposed to crude oil or oil/dispersant as in the righting experiments and the movement trials repeated. Finally dry body weight and test dimensions were measured as already described.

Results

Righting behaviour: Effect of body size on righting behaviour

It is to be expected that the larger an urchin is, the longer it would take to right. To test this premise, total righting time (TT) was plotted against the various body size parameters measured (dry weight), W; maximum test diameter, D; maximum test height, H; and peristome diameter,P) for the two control groups of urchins separately (i.e. urchins subjected to righting trials previous to treatment with oil and urchins subjected to righting trials previous to treatment with oil/BP1100X). The calculated regression equations and the corrresponding Pearson product-moment correlation coefficients for these plots are given below:

oil control:

$TT = 271.4 - 7.428W$ ($r = -0.557$, d.f. = 14, $0.02 < P < 0.05$)
$TT = 364.5 - 93.440H$ ($r = -0.473$, d.f. = 14, $0.05 < P < 0.10$)
$TT = 406.2 - 58.140D$ ($r = -0.552$, d.f. = 14, $0.02 < P < 0.05$)
$TT = 378.4 - 156.662P$ ($r = -0.429$, d.f. = 14, $0.05 < P < 0.10$)

oil/BP1100X control:

$TT = 77.6 + 4.577W$ ($r = 0.545$, d.f. = 14, $0.02 < P < 0.05$)
$TT = 114.9 - 2.076H$ ($r = -0.111$, d.f. = 14, $0.50 < P$)
$TT = 54.9 + 20.829D$ ($r = 0.347$, d.f. = 14, $0.10 < P < 0.20$)
$TT = 27.5 + 75.228P$ ($r = 0.377$, d.f. = 14, $0.10 < P < 0.20$)

Only the correlation between total righting time and body dry weight is significant (at P = 0.05) for both controls. It is however surprising that while in the oil control total righting time is inversely proportional to dry weight, in the oil/BP1100X control it is directly proportional. The only other body size parameter with a significant correlation with total righting time is maximum test diameter, however only in the oil control. Body dry weight therefore appears to be the best descriptor of body size of the four parameters measured.

Dry weight was plotted against total righting time for the two groups of urchins after exposure to oil and oil/BP1100X respectively and regression equations and correlation coefficients calculated as above. The following results were obtained:

treatment = oil

$TT = 177.3 + 0.808W$ ($r = 0.041$, d.f. = 14, $0.50 < P$)

treatment = oil/BP1100X

$TT = 96.3 + 5.485W$ ($r = 0.474$, d.f. = 14, $0.05 < P < 0.10$)

In both cases there is no significant correlation (at P = 0.05) between total righting time and body dry weight in contrast to the two control groups. Treatment with oil and oil/BP1100X therefore appears to affect this relationship. This is particularly true in the case of oil since the relationship was changed from a negative one to positive following exposure to oil.

Total righting time is the sum of latency time (LT) and righting time (RT). The corresponding regression equations and correlation coefficients for the relationship of these behavioural parameters with dry weight for the two treatments are:

oil control:

$LT = 7.63 + 0.039W$ ($r = 0.050$, d.f. = 14, $0.50 < P$)
$RT = 263.80 - 7.467W$ ($r = -0.563$, d.f. = 14, $0.02 < P < 0.05$)

oil:

$LT = 4.27 + 0.165W$ ($r = 0.167$, d.f. = 14, $0.50 < P$)
$RT = 173.00 + 0.643W$ ($r = 0.033$, d.f. = 14, $0.50 < P$)

oil/BP1100X control:

$LT = 4.08 + 0.030W$ ($r = 0.072$, d.f. = 14, $0.50 < P$)
$RT = 73.50 + 4.547W$ ($r = 0.552$, d.f. = 14, $0.02 < P < 0.05$)

oil/BP1100X:

$LT = 8.39 - 0.169W$ ($r = -0.216$, d.f. = 14, $0.20 < P < 0.50$)
$RT = 87.92 + 5.654W$ ($r = 0.488$, d.f. = 14, $0.05 < P < 0.10$)

Latency time is not correlated with body dry weight in either control or after exposure to oil or oil/dispersant. On the other hand, the results for righting time parallel those obtained for total righting time. This is expected since in the whole righting sequence, it is only during righting proper that the urchin has to drag its mass against gravity. What remains unexplained is why the relationship between mass and righting time (and consequently, total righting time also) should be negative in the case of the oil control.

Righting behaviour: effect of oil and oil/Dispersant on righting behaviour

The values of latency time, righting time and total righting time for each sea urchin were converted to a "standard" body dry weight of 13.00 g using the relationship:

$$y = aW^b$$

where y = behavioural parameter, W = body dry weight, and a and b are constants whose value is obtained from a plot of y against W for each data set. Mean values for each behavioural parameter before and after treatment were then calculated (Tables XII and XIII).

Table XII

Paracentrotus lividus. Mean values and standard deviations of latency time (LT), righting time (RT) and total righting time (TT) for a "standard" animal of body dry weight 13.00 g before and after exposure to oil.

	Control			Oil		
	LT(s)	RT(s)	TT(s)	LT(s)	RT(s)	TT(s)
$\bar{x}$	8.25	157.49	165.94	6.60	190.84	197.16
s.d.	3.97	63.75	64.44	4.60	105.62	103.77
n	16	16	16	16	16	16

The raw data from which the values in Tables XII and XIII were calculated were compared statistically using Student's t-test to test for differences in means. No significant differences (at P = 0.05) were found between mean latency time, mean righting time and mean total righting time before and after exposure to oil (in all cases $0.20 < P < 0.50$). Therefore, exposure to oil does not appear to have an effect on the righting response of *P. lividus* under the experimental conditions employed in this study.

Table XIII

Paracentrotus lividus. Mean values and standard deviations of latency time (LT), righting time (RT) and total righting time (TT) for a "standard" animal of body weight 13.00 g before and after exposure to oil/BP1100X.

	Control			Oil/BP1100X		
	LT(s)	RT(s)	TT(s)	LT(s)	RT(s)	TT(s)
$\bar{x}$	4.48	134.95	139.52	6.00	164.31	170.52
s.d.	2.05	32.25	33.23	3.60	47.33	48.03
n	16	16	16	16	16	16

In the case of the oil/BP1100X experiment, no significant differences (at $P = 0.05$) were found between mean latency time before and after exposure to oil/dispersant, however, mean righting time, and consequently, mean total righting time were found to be significantly longer following exposure to oil/dispersant ($0.02 < P < 0.05$ in both cases). Mean righting time shows a 21.8% increase while mean total righting time shows a 22.2% increase following exposure to oil/BP1100X mixtures.

Movement: directed movement

Between trials on each urchin, the experimental aquarium was emptied and the bottom scrubbed as a precaution against laying down of mucus or other trials by the urchin which could affect subsequent urchins. Also, since P. lividus are photonegative, the lighting on the experimental aquarium was adjusted to give an even illumination over the entire bottom area. To test whether these precautions were sufficient to prevent preferential movement towards some part of the aquarium and to test whether exposure to oil and oil/dispersant had any effect on direction of movement, the number of times an urchin ended up in the four corner areas (a square of side 18 cm at each corner) was counted (Table XIV).

Table XIV

Paracentrotus lividus. The number of times animals ended in the top left (TL), top right (TR), bottom left (BL) and bottom right (BR) 18 x 18 cm areas at the corners of the experimental aquarium during movement trials.

Treatment	TL	TR	BL	BR
oil control	6	6	11	9
oil	9	8	16	6
oil/BP1100X control	7	16	12	12
oil/BP1100X	10	10	16	12

Goodness-of-fit X^2 analyses showed that in no case was the distribution of urchins significantly different (at P = 0.05) from 1:1:1:1. Contingency X^2 analyses showed that there was no significant difference in the distribution of urchins in the four corners between oil control and oil (0.05 P 0.75) and oil/BP1100X control and oil/BP1100X (0.25 P 0.50). P. lividus therefore do not appear to show any directed response in the experimental aquarium irrespective of treatment.

Effect of body size on movement

Speed of movement (S) and rate of turning (RT) were plotted against body dry weigt (W) and regression equations and correlation coefficients calculated:

oil control:

$S = 1.15 + 0.110W$ ($r = 0.441$, d.f. = 14, $0.05 < P < 0.10$)
$RT = 0.096 + 0.006W$ ($r = 0.400$, d.f. = 14, $0.01 < P < 0.20$)

oil:

$S = 1.85 + 0.089W$ ($r = 0.507$, d.f. = 14, $0.02 < P < 0.05$)
$RT = 0.150 + 0.003W$ ($r = 0.235$, d.f. = 14, $0.20 < P < 0.50$)

oil/BP1100X control:

$S = 4.75 - 0.048W$ ($r = -0.253$, d.f. = 14, $0.20 < P < 0.50$)
$RT = 0.20 + 0.003W$ ($r = 0.293$, d.f. = 14, $0.20 < P < 0.50$)

oil/BP1100X:

$S = 4.37 - 0.026W$ ($r = -0.135$, d.f. = 14, $0.50 < P$)
$RT = 0.30 - 0.004W$ ($r = -0.439$, d.f. = 14, $0.05 < P < 0.10$)

The relationship between body dry weight and movement parameters measured appear to be very weak and in all cases irrespective of treatment, the only significant correlations being between speed and dry weight following exposure to oil.

Effect of oil and oil/dispersant on movement

Values for speed and rate of turning obtained were converted for a "standard" urchin of body dry weight 13.00 g as already described and means calculated (Tables XV and XVI).

These means were tested for differences using Student's t-test. No significant difference (at P = 0.05) was found between either mean speed or mean turning rate before and after treatment in both the oil experiment and the oil/dispersant experiment. Exposure to oil alone or oil/BP1100X mixtures does not seem to affect the movement parameters studied here under the experimental conditions employed.

Table XV

Paracentrotus lividus. Mean values for speed of movement (S) and rates of turning (RT) for a "standard" animal of body dry weight 13.00 g before and after exposure to oil.

	Control		Oil	
	S (cm min^{-1})	RT (turns min^{-1})	S (cm min^{-1})	RT (turns min^{-1})
$\bar{x}$	2.65	0.165	3.00	0.177
s.d.	0.96	0.065	0.68	0.071
n	16	16	16	16

Table XVI

Paracentrotus lividus. Mean values for speed of movement (S) and rate of turning (RT) for a "standard animal of body dry weight 13.00 g before exposure to oil/BP1100X.

	Control		Oil/BP1100X	
	S (cm min^{-1})	RT (turns min^{-1})	S (cm min^{-1})	RT (turns min^{-1})
$\bar{x}$	4.07	0.222	4.01	0.243
s.d.	0.93	0.067	0.93	0.042
n	16	16	16	16

3.5.4 Conclusions

Significant mortalities on exposure to relatively high levels of chemically dispersed PHC were recorded which moreover were dependent on the type of dispersant applied. Of the dispersants investigated, only DISPOLENE 32S significantly altered the respiratory activities of sea urchins exposed to chemically dispersed PHC. A significant increase in righting time indicating interference with the highly co-ordinated activities of spines and tube fee, was recorded. No significant effect on locomotor activities were recorded at the relatively low levels of chemically dispersed PHC investigated.

3.6 Palaemon elegans (CRUSTACEA : DECAPODA)

3.6.1 Toxicity of oil and oil dispersants

Materials and Methods

Shrimps were collected from shallow water (0-1m) from various bays round the coast of Malta, transferred to the laboratory and allowed to acclimatize to laboratory conditions for at least two days before use in toxicity experiments. Shrimps were used within 10 days of collection.

The exposure apparatus used was the same as that already described in Section 3.3.1 except that instead of eight glass funnels, the exposure chambers consisted of four circular "Perspex" aquaria having internal diameter 35.5 cm and filled to a depth of 15 cm with water. All experiments were carried out at an ambient temperature of 20 $^{o}C \pm 1$. In all experiments exposure time of the test organism to the pollutant was 100 minutes.

The experimental precedure adopted is the same as that described in Section 3.3.1 except that 10 shrimps were placed in each exposure chamber. Crude oil was added at a nominal initial concentration of 1 cm^3 l^{-1}. Two dispersants were tested: BP1100X added at a nominal initial concentration of 1 cm^3 l^{-1} five minutes after addition of crude oil, and DISPOLENE 32S first diluted 1:9 with sea water to produce a 10% solution as per the manufacturer's instructions and then this solution added to the exposure apparatus to give a nominal initial concentration of diluted dispersant of 1 cm^3 l^{-1}. Mortalities were determined 24 hours and 48 hours after removal of the test animals from the exposure apparatus.

Results

Table XVII shows mortalities recorded after exposure to oil, oil/BP1100X and oil/DISPOLENE 32S. Each of the mortalities in the replicate experiments reported in Table XVII is itself the sum of mortalities recorded in each of the four exposure chambers used in each run. Contingency X^2 testing of the mortalities recorded in these four replicate chambers showed no significant differences (at $P = 0.05$) between them and these results were therefore pooled. The only exception was in the case of oil/BP1100X replicate 1 where one exposure chamber suffered 80% mortality as opposed to 10%, 0% and 10% for the other three, making mortality in the first chamber very significantly different ($P < 0.001$) from that in the others. The reasons for this discrepancy are not known, however, it was thought safer to discard the results from this chamber. The values for oil/BP1100X replicate 1 reported in Table XVII are those for the three chambers for which mortalities were comparable only.

The null hypothesis that mortalities after 48 hours in the two replicate oil experiments are not significantly different was tested and similarly for the two replicate oil/BP1100X and oil/DISPOLENE 32S experiments. In no case was a significant difference found (contingency X^2, $P = 0.05$) and the corresponding replicates were pooled.

Table XVII

Palaemon elegans. Mortalities after 24 h and 48 h following exposure to oil and oil dispersant mixtures. Percent mortalities are shown in parenthesis.

Treatment	Initial number of shrimps	Number dead after 24 h	Number dead after 48 h
oil:			
replicate 1	32	2 (6.3%)	3 (9.4%)
replicate 2	40	2 (5.0%)	4 (10.0%)
oil/BP1100X:			
replicate 1	30	0 (-)	2 (6.7%)
replicate 2	40	3 (7.5%)	10 (25.0%)
oil/DISPOLENE 32S:			
replicate 1	40	3 (7.5%)	5 (12.5%)
replicate 2	40	3 (7.5%)	12 (30.0%)

No significant difference in mortality after 48 hours was found between shrimps exposed to oil alone and oil/BP1100X (X^2 = 1.107, $0.25 < P < 0.50$) and similarly for oil and oil/DISPOLENE 32S (X^2 = 2.970, $0.05 < P < 1.10$). No significant difference was found in mortalities after 48 hours for shrimps exposed to oil/BP1100X and oil/DISPOLENE 32S (X^2 = 0.183, $0.1 < P < 0.25$). It therefore appears that exposure to crude oil alone or oil dispersed with either BP1100X or DISPOLENE 32S produces the same mortalities in P. elegans under the experimental conditions employed in this study.

Mortalities after 24 hours were very similar for all three treatments but they deviate after 48 hours, the sharpest increase being for DISPOLENE 32S. Table XVIII shows the overall mortalities recorded.

Table XVIII

Palaemon elegans. Percent mortalities recorded after exposure to crude oil and oil/dispersant mixtures.

Treatment	% Mortality	
	After 24 h	After 48 h
oil	5.6	9.7
oil/BP1100X	4.3	17.1
oil/DISPOLENE 32S	7.5	21.3

3.6.2 Conclusions

Exposure to chemically dispersed PHC produced significantly higher mortalities than mechanically dispersed PHC after 48 hours recovery, the effect being dependent on the type of dispersant used. This work is not complete yet. At present, other dispersants as well as the same dispersants at different temperatures are being investigated.

3.7 Clibanarius erythropus (CRUSTACEA : DECAPODA)

3.7.1 The effects of oil and oil dispersants on food attraction behaviour

Materials and Methods

Hermit crabs were collected from shallow water (0-1 m) from Bahar ic-Caghaq, Malta and transported to the laboratory where they were allowed to acclimatize for at least three days prior to use in experiments.

The testing apparatus was very similar to that used by Schembri (1981) and consisted of a glass observation tube (length 22 cm x diameter 4 cm) connected at one end to a tank (15 x 1.25 x 16.5 cm) in which sea water continuously flushed away water from the observation tube (Fig.11). The other end of the observation tube was fitted with a nozzle connected to a constant head device via two identical glass test tubes one of which contained food (fish tail muscle) and the other was "clean" and acted as control. The water flow could be switched from one test tube to the other by means of a 3-way tap. The bottom of the observation tube was covered with a 1.5 cm thick layer of set cement to simulate the natural rocky substratum of the crabs. The observation tube was marked 10 cm from the nozzle end. The whole apparatus was screened and illumination was provided by a red 15W lamp producing a luminous intensity of 25 lux at the observation tube. The flow rate through the nozzle was approximately 1 cm^3 5^{-1} and dye tracers were used to check that this produced a laminar flow down the middle of the observation tube.

Hermit crabs were starved in clean sea water for two days prior to use. An individual crab was then placed at the open end of the observation tube and the "clean" water current turned on. The response of the crab to the current, the direction of movement and the time taken to reach the nozzle after passing the 9 cm mark were noted. The crab was then replaced at the start position and the "food" current turned on and the behaviour of the crab again noted as before. The manner in which the crab responded to the current was scored as "direct", "random" or "no movement" as follows: a "direct" response was movement directly towards the nozzle without any erratic or meandering movements in the observation tube; a "random" response was movement towards the nozzle but in an erratic manner involving many stops and starts and backwards and forwards movements in the tube; "no movement" was non-directed movement in which the crab either dragged the shell in circles but did not move more than two body lengths away from its start position, or else did not move at all. Trials in which the crab showed a "no movement" response for a continuous period of 5 minutes were stopped after that time.

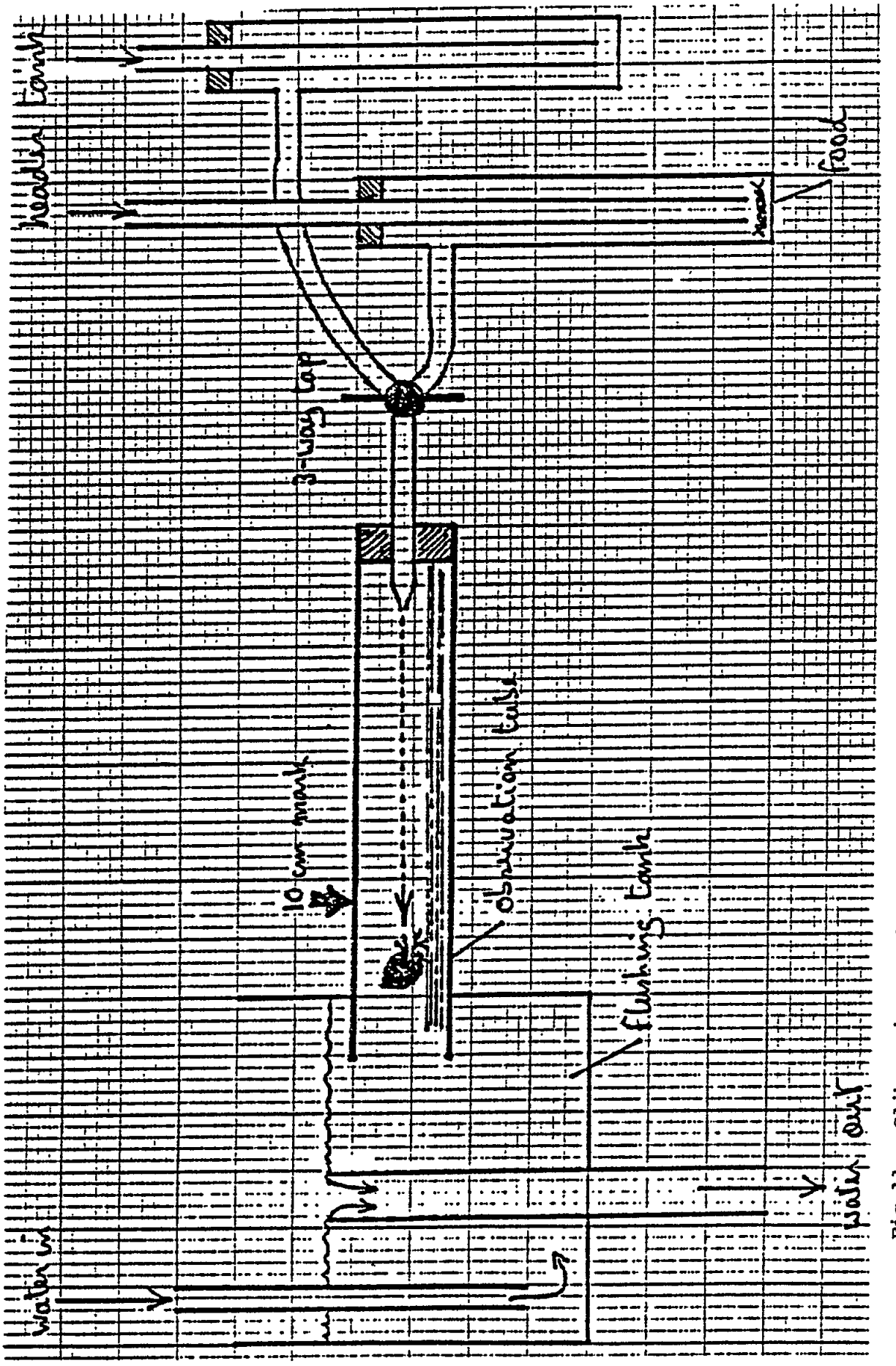

Fig.11 Clibanarius erythropus. Set-up to investigate food detection (Section 3.7.1)

Three batches of crabs were tested. One batch was not exposed to any pollutant and served as the control group. A second batch was exposed for 24 hours to crude oil at a nominal initial concentration of 0.01 cm^3 l^{-1}. The third batch was dosed with oil at the same concentration as the second batch but after 1 minute it was dosed with BP1100X at a nominal initial concentration of 0.01 cm^3 l^{-1} and left in this mixture for 24 hours. All exposures were made in the apparatus described in Section 3.5.2 and under the same ambient conditions.

Results

Levels of PHC in the test oil mixtures dropped from 2.2 to 0.15 ppm, and in the oil/dispersant mixtures from 2.7 to 0.27 ppm during the 24 hour exposure period.

Table XIX shows preliminary results of food detection experiments on C. erythropus. Because of the low number of replicates, the following statistical analyses are not strictly valid.

Contingency X^2 testing showed no significant difference (at P = 0.05) between the number of crabs responding to "clean" and "food" by moving towards the nozzle in the control group, and similarly for the oil and oil/dispersant groups. Neither was the number of crabs responding to "clean" significantly different (at P = 0.05) in the control, oil and oil/dispersant groups, and similarly for number responding to "food".

Table XIX

Clibanarius erythropus. Food detection abilities on exposure to oil and oil/dispersant mixtures. Responses were scored as "direct" (D), "random" (R) or "no movement" (NM); for explanation of terms, see text

	Control group		Oil group		Oil/BP1100X group	
	Clean	Food	Clean	Food	Clean	Food
No. tested	20	20	20	20	20	20
No. moving towards nozzle	2	8	6	9	8	3
No. reaching nozzle	2	4	6	8	6	3
Response D	2	4	3	6	3	0
R	0	0	3	2	3	3
NM	0	0	0	0	1	2
Mean time to reach nozzle (s)	32.75	34.00	51.20	31.94	40.83	46.33
s.d.	11.67	11.56	27.34	9.73	16.23	15.57
n	2	4	5	8	6	3

The number of crabs which reached the nozzle in "clean" and "food" was not significantly different (at P = 0.05) for the control group and similarly for the oil and oil/dispersant groups. Neither was there any significant difference (at P = 0.05) in the numbers reaching the nozzle in "clean" in any of the three groups, and similarly for "food".

The number of "direct", "random" and "no movement" responses was not significantly different (at P = 0.05) between "clean" and "food" in any group. The number of "direct", "random" and "no movement" responses in "clean" was not significantly different (at P = 0.05) in any group, however a significant difference was found for "food" ($0.001 < P < 0.025$) showing that the response pattern to fish odour was different in the three groups. This difference is due to the response pattern in crabs exposed to oil/BP1100X which show no "direct" responses but do show "no movement" responses in direct contrast to the situation for either the control group or the oil group. The difference in response pattern when tested with "food" is not significant (at P = 0.05) in the control and oil groups but is very significantly different between the oil/dispersant and the other two groups combined ($0.001 < P < 0.005$).

No significant difference (at P = 0.05) was found in time taken to reach the nozzle between "clean" and "food" for any of the three groups (Mann-Whitney U-test). No significant difference (at P = 0.05) was found between time taken to reach the nozzle for "clean" in the three batches and similarly for "food" (Kruskal-Wallace one-way analysis of variance by ranks).

3.7.2 Conclusions

These preliminary data indicate that relatively low concentrations of chemically dispersed PHC interfere with the food detection activities of this species. No such effect was evident on exposure to mechanically dispersed PHC. Since the actual levels of PHC in both cases were approximately equal, these results may not be explained simply due to higher concentrations of PHC available to test animals during the exposure period in the case of the more stable oil/dispersant mixtures. The presence of the dispersant itself may be directly affecting this biological response. Further work is in progress to clarify and confirm these results.

4. DISCUSSION

All the dispersants tested are reported to be of low toxicity to marine life by their manufacturers. Several investigations (e.g. Swedmark et al., 1973; Dodd, 1974) have indeed proved that such "new" generation of dispersants are relatively much less toxic than the older generation of dispersants, mainly due to their non-aromatic solvent base. In this study, exposure to such dispersants alone did not generally cause any biological effect which was significantly different from exposure to oil alone (e.g. mortality in Patella caerulea), though an increase in the respiratory activities of Patella caerulea on exposure to FINASOL OSR7 alone was recorded.

However, in assessing the likely ecological impact of applying oil dispersants on crude oil spilled in the field, it was thought more reasonable to investigate and compare the biological effects caused by chemically dispersed with those of mechanically dispersed crude oil. As pointed out recently (Royal Society, 1980) "dispersants will remove oil from the water surface and extend to greater depths the level of the body of water containing

available oil droplets and dissolved or accommodated PHC, with the risk that acutely toxic levels will reach lower down in the water column". This increase in bio availability of spilt oil by the application of dispersants will be expected to be more important in inshore, shallow coastal areas where dilution of the dispersed oil is limited and access to benthic organisms facilitated. Such areas (e.g. many harbours in the Mediterranean) are often exposed to periodic or chronic pollution by oil and it is generally the practice to apply dispersants repeatedly to maintain the sea surface relatively clean from oil. In these cases, relatively high levels of dispersed PHC may be expected in the water column. Therefore we consider the test concentrations of chemically or mechanically dispersed oil used throughout the present investigations to be "ecologically realistic".

Test animals were moreover exposed to rapidly decreasing levels of pollutants as would be expected to take place at the sea due to the natural dispersion of spilt oil. Furthermore, in test exposures simulating beach conditions, weathered crude oil was used. All physiological and behavioural responses of exposed animals investigated in this study may be considered to be biologically and ecologically significant and alterations in such responses may affect the organism's overall adaptive strategy.

In the majority of investigations undertaken, chemically dispersed PHC produced greater mortalities and altered the several physiological and behavioural activities of the range of species tested, more significantly than oil alone. This was probably due to higher levels of PHC in the more persistent and stable chemically dispersed oil emulsions. Furthermore, other environmental stresses such as temperature were shown to enhance the toxic effects of oil and dispersant mixtures. (Phaeodactylum tricornutum growth rates). As indicated in the text, several investigations are not yet terminated and further results are expected to clarify and confirm these conclusions.

5. ACKNOWLEDGEMENTS

Main collaborator : Patrick J. Schembri

Other collaborators: N. Aquilina, K. Bergman, H. Bonanno, N. Camilleri, P.H. Camilleri, D. Chetcuti, J.C. De Monaco, C. Galea, K. Hubbeling, M. Pace, M. Sammut, A. Vella Catalano.

We wish to acknowledge the help of: The Regional Oil Combatting Centre of Manuol Island, for identification of dispersants in most common use in the Mediterranean; The Kuwait Petroleum Corporation, for supplying the test crude oil samples; the various dispersant manufacturers, for supplying the required test samples of the dispersants investigated; J. Debono for collection of test animals; J.J. Drury, S. Cachia and Y. Grima for typing the final manuscript.

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RESEARCH ON THE EFFECTS OF OIL DISPERSANTS ON MARINE ORGANISMS

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A B S T R A C T

The acute toxicity of oil dispersants BP1100X and BP1100WD (British Petroleum) as well as that of MXG-2 and M-2 (INA-Yugoslavia) to juvenile stages of mullet Liza saliens was tested. The acute toxicity of BP1100WD to development stages of sea bass (Dicentrarchus labrax) was also examined.

Tests were carried out under static conditions with aeration, but in the case of MXG-2 and M-2, acute toxicity tests were also carried out under conditions with no aeration.

BP1100X and BP1100WD are very toxic to juveniles of Liza saliens. Lethal concentration threshold of BP1100X for 48 h exposure is slightly below or close to the 100 $\mu l\ l^{-1}$ concentration, whereas for BP1100WD it is above 100 $\mu l\ l^{-1}$.

MXG-2 and M-2 dispersants strongly affect dissolved oxygen level in the tanks. Threshold of LC_{50} of MXG-2 dispersant for 96 h exposed Liza saliens with aeration is below 100 $\mu l\ l^{-1}$ concentration. At lower concentrations MXG-2 decreases median lethal time in non-aerated media in relation to aerated ones. At higher MXG-2 concentrations concentration/response curves are almost identical irrespective of the experimental conditions. Dispersant M-2 shows some differences in toxicity in aerated tanks. In non-aerated tanks at lower M-2 concentrations where the dissolved oxygen content was drastically reduced, Liza saliens mortality was lower than in aerated tanks.

Low mortality of sea bass eggs shows that they are resistant to the effects of BP1100WD. However, spinal deformities were recorded in some hatched larvae. BP1100WD concentrations with LC_{50} for 96 h show a successive increase for postlarvae, larvae and juveniles of Dicentrarchus labrax with the respective values 64, 140 and 1400 $\mu l\ l^{-1}$.

1. INTRODUCTION

Oil dispersants are used for dispersing oil from the surface of the sea and cleaning coastlines after oil spills. Basically they consist of a mixture of a surfactant, a hydrocarbon solvent and sometimes, of a stabilizing agent. Their toxicity may depend on the type of solvent (Portmann and Connor, 1968), surfactant (Swedmark et al., 1971) or on the synergistic effects between the surfactant and the solvent (Nagell et al., 1974; Norton and Franklin, 1980).

It is generally well known that younger (earlier) stages are more susceptible to toxic effects (Mironov, 1972; Kühnold, 1977). Thus, whereas some organisms are capable of detecting oil dispersants and of avoiding them (Portmann, 1972; Gyllenberg and Lundqvist, 1976) fish eggs are passive and therefore exposed to strong pollutant effects in the sea water.

The data on the effects of oil dispersants on the Mediterranean marine organisms are relatively scarce. It seems that the toxicity of dispersants to marine organisms is much higher in the Mediterranean than in the more northern seas. The scope of this work is, in the first place, to establish the toxicity of oil dispersants to some marine organisms from the Adriatic Sea.

2. MATERIAL AND METHODS

Experimental methods for Liza saliens

The tests of acute toxicity of oil dispersants BP1100WD and BP1100X (British Petroleum) as well as of MGX-2 and M-2 (INA-Yugoslavia) to juvenile mullet Liza saliens was carried out under static conditions (Portmann and Connor, 1968). Oil dispersants BP1100WD and M-2 were diluted with sea water (1:10) before toxicity tests.

Acute toxicity of dispersants BP1100WD and BP1100X was tested on Liza saliens individuals of 4.0 $\pm$ 0.6 cm ($\bar{x}$ $\pm$ SD) length and 0.57 $\pm$ 0.26 g weight. Ten Liza saliens were added to each 20 litre tank filled with sea water. The tests were carried out in aerated sea water at 20 ^{o}C in a thermostatic chamber.

Toxicity of MXG-2 and M-2 oil dispersants was tested in both aerated and non-aerated tanks and control tanks. Ten fish of 3.1 $\pm$ 0.8 cm length and 0.25 $\pm$ 0.29 g weight were added to 20 litre tanks filled with sea water. The temperature of the sea water was kept at 18 ^{o}C in the thermostatic chamber. Dissolved oxygen concentration was recorded by Winkler's method every 24 h.

During the tests, fish were kept at 12 hour light-darkness photoperiod. Mortality was recorded at determined intervals.

Median lethal time (LT_{50}) (Franklin, 1980) was read by plotting cumulative mortality for each concentration of oil dispersant against the time logarithm on probit paper. Median lethal concentrations (LC_{50}) and 95% confidence limits for determined exposure time were obtained by probit analysis.

Experimental methods for Dicentrarchus labrax

The method of induced sea bass (Dicentrarchus labrax) spawning and rearing of different stages were described earlier (Katavic, 1984).

Tests of acute toxicity of BP1100WD to eggs, larvae, postlarvae and juveniles of sea bass were carried out under static conditions with medium basin aeration. Five different concentrations of oil dispersant with controls were used in 96 h tests which were duplicated. Eggs, larvae and postlarvae were treated in one litre volumes so that 100 eggs and larvae respectively and 20 postlarvae were placed in each jar. Ten juveniles were placed in each 10 litre volume of sea water with dispersant.

Newly fertilised eggs, one day old larvae, 17 day old postlarvae and five month old juveniles with an average weight of 0.89 g were treated in the dispersant.

BP1100WD was mixed with sea water in the ratio 1:10 before use, as recommended by the manufacturer. Test concentrations, thus, refer to this mixture.

Temperature was kept constant by water thermostats. The temperature was 15.5 ± 0.2 °C in the experiments with eggs, larvae and postlarvae and 21 ± 1 °C in the experiment with juveniles. No food was supplied during experiments. Salinity of the sea water ranged from 36.5 to 38.0 x 10^{-3}. Oxygen content was observed daily. Oxygen concentration was never lower than 90% in the experiments with eggs, larvae and postlarvae whereas it reached 75% not earlier than the fourth day in the experiment with juveniles. Experiments were carried out at photoperiod of 12 hours.

Mortality was 18% in the controls with eggs and 10% in those with larvae. Therefore, the mortality correction (Abbot, 1925) was applied for the calculation of LC_{50}. LC_{50} and 95% confidence limits were determined for each exposure time by the probit method of Bliss (Fisher and Yates, 1949; Stora, 1974).

3. RESULTS

Acute toxicity of BP1100WD and BP1100X to Liza saliens

The test of acute toxicity of BP1100WD and BP1100X oil dispersants continued for 48 h of fish exposure to concentrations ranging from 10-1000 $\mu g\ l^{-1}$. BP1100X showed high toxicity to juvenile mullet Liza saliens. No fish mortality was recorded at 10 $\mu g\ l^{-1}$ dispersant concentration. Concentration/response curve (Fig.1) shows that the threshold of lethal concentration for 48 h exposure is somewhere between 10 $\mu l\ l^{-1}$ and 100 $\mu l\ l^{-1}$ BP1100X. Sigmoid curve shape may be indicative of two distinct toxicity components of BP1100WD.

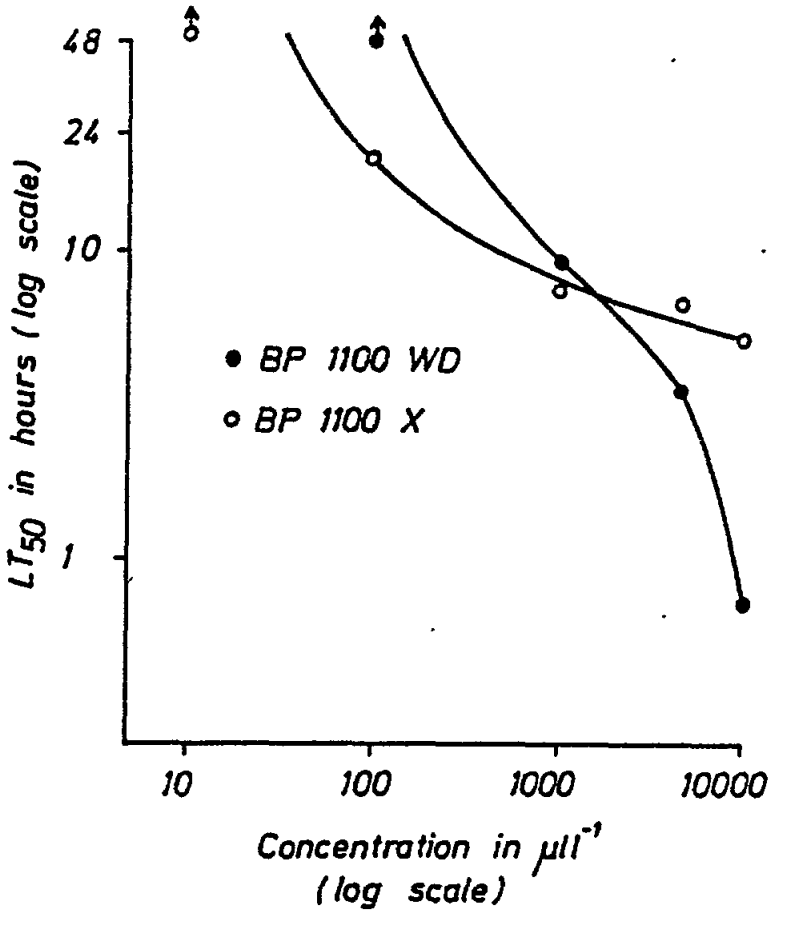

Fig.1 Toxicity curves of BP1100WD and BP1100X to juvenile Liza saliens

Acute toxicity of MXG-2 and M-2 to Liza saliens

At lower concentrations the toxicity of MXG-2 oil dispersant in aerated tanks differed from that in non-aerated tanks. Concentration/response curve (Fig.2) shows that LC_{50} threshold of MXG-2 dispersant was lower than 100 µl l^{-1} for the 96 h of fish exposure with aeration. At low concentrations MXG-2 dispersant with no aeration causes reduction of median lethal time (Fig.2). This reduction of the time of fish survival may also be due to the lower level of dissolved oxygen.

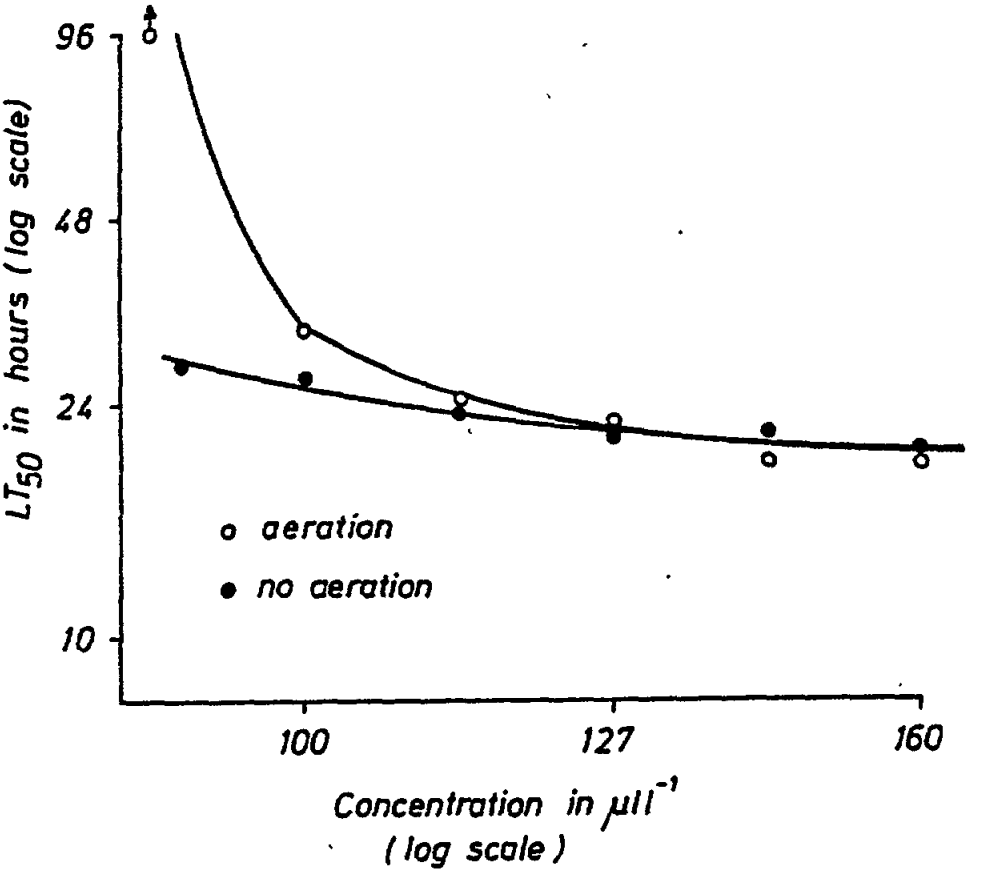

Fig.2 Toxicity curves of MXG-2 to juvenile Liza saliens under aerated and non-aerated conditions

Oil dispersant MXG-2 strongly affects dissolved oxygen content in the tanks (Fig.3). The greatest reduction in oxygen saturation was recorded in aerated tanks after 24 h and in non-aerated tanks after 48 h. Very low level of dissolved oxygen in non-aerated tanks may affect the time of fish survival particularly at lower concentrations. At concentrations of MXG-2 exceeding 100 µl l^{-1} some portions of concentration/response curves are almost overlapping, irrespective of the level of dissolved oxygen.

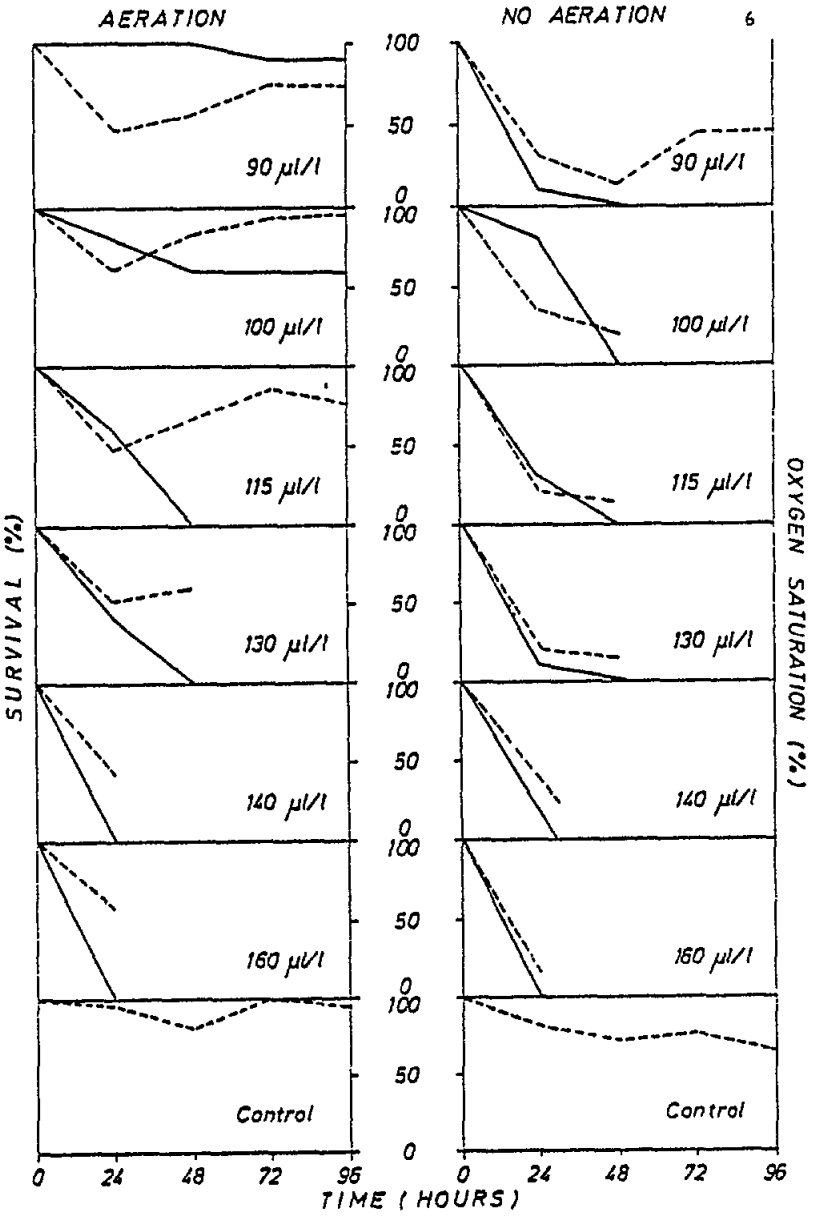

Fig.3 Survival of juvenile Liza saliens (unbroken line) and dissolved oxygen level (broken line) for the time of the experiment with different MXG-2 concentrations in aerated and non-aerated tanks

LC_{50}, LC_{90} and 95% limits for 19, 24 and 32h of fish exposure to MXG-2 dispersant were calculated from Liza saliens mortality (Table I).

Table I

Median lethal concentration (LC_{50}) of MXG-2 and concentrations lethal to 90% (LC_{90}) of juvenile Liza saliens (95% confidence limits are given in brackets).

LC	Aeration µl l^{-1}	No aeration µl l^{-1}
$LC_{50}/19$	157 (133,187)	157 (137,180)
$LC_{50}/24$	112 (106,118)	108 (102,114)
$LC_{50}/32$	100 (96,104)	-
$LC_{90}/19$	228 (157,330)	208 (155,280)
$LC_{90}/24$	131 (118,145)	125 (115,136)
$LC_{90}/32$	115 (107,124)	

Effects of toxicity of lower concentrations of oil dispersant M-2 on Liza saliens mortality in aerated tanks differed from those in non-aerated tanks (Fig.4).

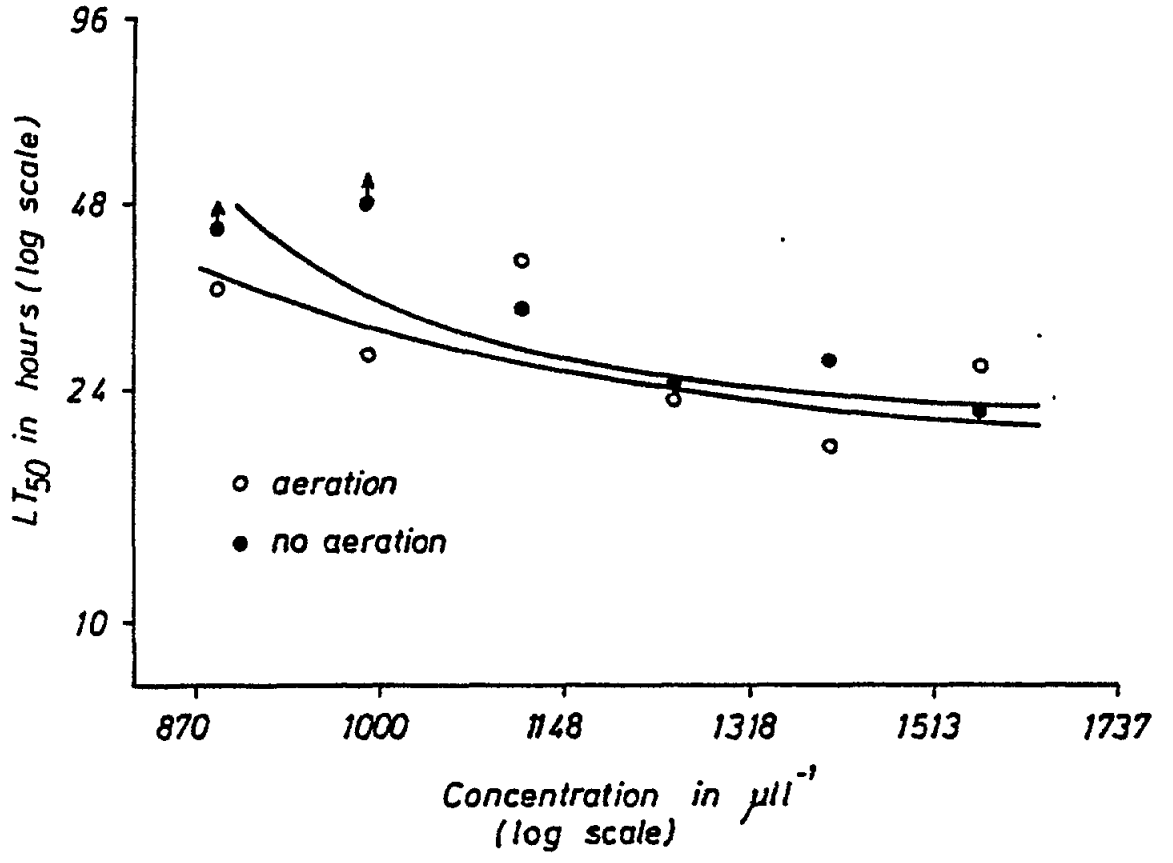

Fig.4 Toxicity curves of M-2 to juvenile Liza saliens in aerated and non-aerated tanks

Oil dispersant M-2 also affects the level of dissolved oxygen in the tanks (Fig.5). Thus, whereas lower concentrations of M-2 in aerated tank were lethal to 100% on the fish after 48 h exposure, they were lethal to 60% of the fish in non-aerated tanks with lower dissolved oxygen level for the same exposure time (Fig.5).

Values of LC_{50} and LC_{90} of M-2 oil dispersant to juvenile Liza saliens are given in Table II.

Table II

Median lethal concentration (LC_{50}) of M-2 and concentrations lethal to 90% (LC_{90}) of juvenile Liza saliens (95% confidence limits are given in brackets).

LC	Aeration $\mu l\ l^{-1}$	No aeration $\mu l\ l^{-1}l$
$LC_{50/24}$	1271 (1202,1344)	1317 (1216,1426)
$LC_{50/32}$	791 (616,1015)	1027 (909,1160)
$LC_{50/48}$	-	901 (781,1039)
$LC_{90/24}$	1462 (1316,1626)	1718 (1559,1893)
$LC_{90/32}$	1066 (919,1237)	1415 (1115,1796)
$LC_{90/48}$	-	1206 (1017,1430)

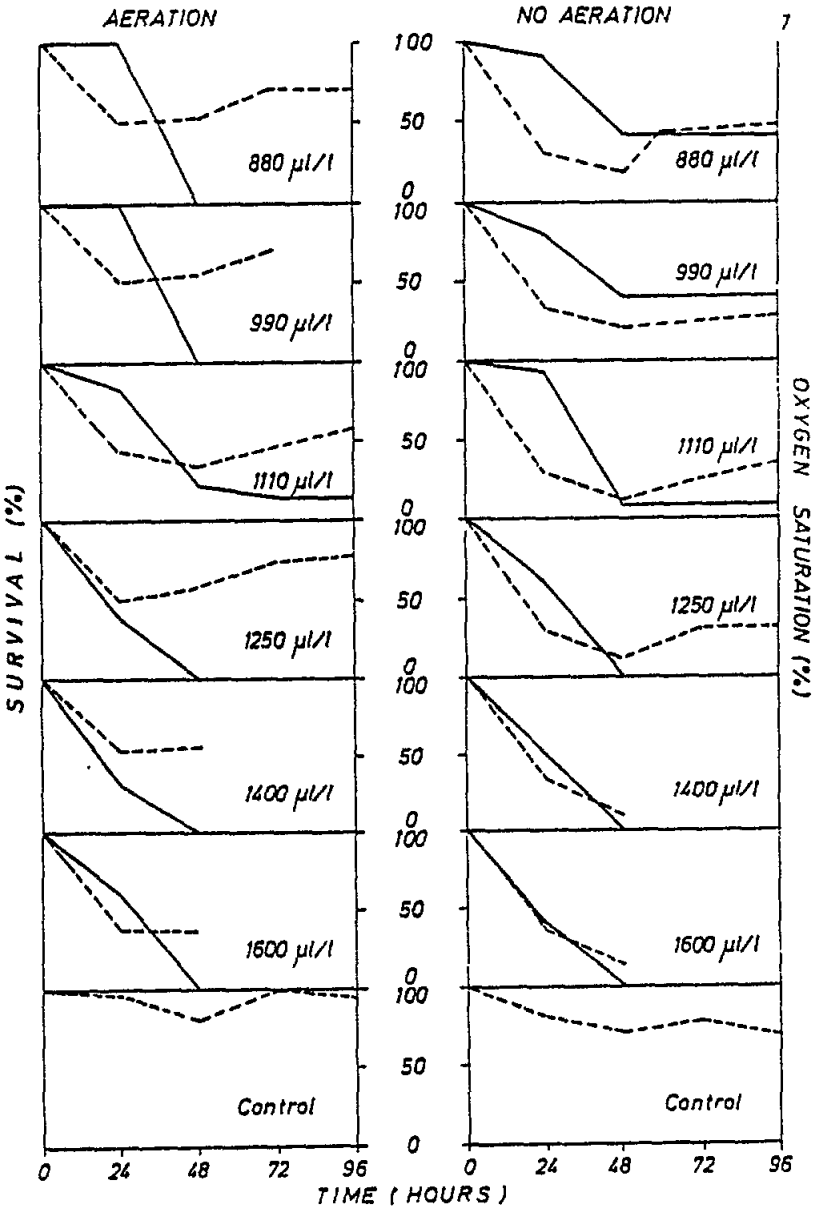

Fig.5 Survival of juvenile Liza saliens (unbroken line) and dissolved oxygen level (broken line) for the time of the experiment with different M-2 concentrations in aerated and non-aerated tanks

Figs.3 and 5 show that adding oil dispersants MXG-2 and M-2 to the sea water causes a decrease of concentrations of dissolved oxygen in the tanks.

Measurement of biological oxygen demand (BOD) in 150 µl l^{-1} MXG-2 and 1500 µl l^{-1} M-2 solution in non-filtered sea water showed no changes in dissolved oxygen for the first ten hours. After 24 h dramatically low level oxygen concentration (36% of saturation) was recorded for both dispersants. After five days no oxygen at all was recorded from BOD bottles. In this time concentration of controlled bottles was dropped on 70% oxygen saturation.

Acute toxicity of BP1100WD to Dicentrarchus labrax

Sea bass eggs are relatively very resistant to BP1100WD effects. After 24-hour exposure egg survival gradually decreased with higher dispersant concentrations (Fig.6). Dead eggs showed the survival of 70% in all concentrations compared with 82% in controls. Embryos begin to develop in eggs in this phase.

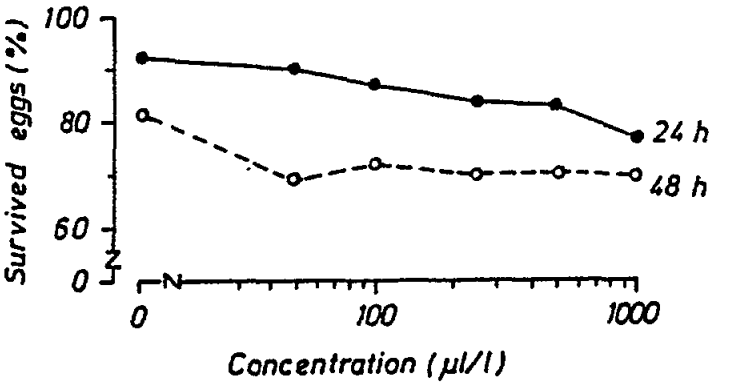

Fig.6 Survival of sea bass eggs after 24 and 48-hour exposures to BP1100WD

Hatching of larvae started during the third day of incubation. Hatching was observed for 5 hours. The beginning of hatching and the time to hatch 50% of the population were obtained from the cumulative percentage of hatched larvae and time by means of probit plots (Table III).

Table III

Differences in the beginning of hatching between controls and BP1100WD and the time to hatch 50% of larvae.

Concentration µl l^{-1}	Beginning of hatching (h)	Time to 50% hatch (h)
0	0	7.5
50	+1.4	5.4
100	+1.1	6.0
250	+1.0	4.8
500	+1.0	5.3
1000	+4.5	4.2

Concentrations up to 500 µl l^{-1} delayed the beginning of hatching by up to an hour in relation to the controls. This delay of the beginning of hatching of about 4.5 h in concentrations of 1000 µl l^{-1} seems to be important with respect to the relatively short embryonic development of sea bass (Katavic, 1984). The mean time to hatch was shortened (80-56%) in all BP1100WD concentrations compared with controls.

After 90-hour exposure hatching success showed no significant differences between different BP1100WD concentrations (Fig.7). Dead eggs found after this period were in the final phase of embryogenesis.

Some changes in larval behaviour were observed in different dispersant concentrations. Larvae were given a slight stroke with a metal needle to observe their mobility. Larval mobility was estimated as normal when the stroke evoked immediate reaction and quick escape as far as possible from the source of stimulus, mild when they escaped 1-3 cm and poor when they reacted with a few twitches.

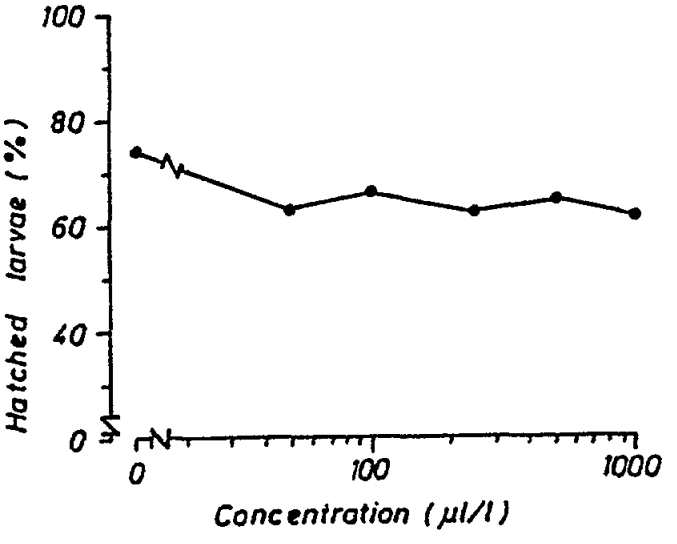

Fig.7 Percentage of hatched larvae after 90-hour exposure to BP1100WD

Thus the mechanically provoked mobility of larvae was gradually reduced by the increase of BP1100WD concentrations and the percentage of spinal deformities increased (Table IV).

Table IV

Effects of BP1100WD on the mobility and morphological characteristics of hatched larvae.

Concentration ($\mu l\ l^{-1}$)	Mobility	Spinal deformities (%)
0	Normal	0
50	Normal	0
100	3-5 cm	27.3
250	1-3 cm	28.6
500	3-4 twitches	50.0
1000	None	26.7

Larvae hatched in 1000 $\mu l\ l^{-1}$ concentration showed no mechanically provoked mobility. Measured heartbeat frequency showed 53±0 (± SD, n=3) heartbeats min^{-1} in these larvae whereas in normal larvae it was 94±13 (± SD, n=6), heartbeats min^{-1}.

The 96-hour exposure to the BP1100WD concentrations of 5-100 $\mu l\ l^{-1}$ caused no significant differences in the mortality between one day old larvae and controls. The degree of yolk sac resorption was measured after 48-hour exposure of larvae. BP1100WD increases the rate of yolk sac resorption (Table V).

Table V

Yolk sac resorption in larvae (taken as the surface area of an ellipse) exposed to BP1100WD for 48-hours.

Concentration ($\mu l\ l^{-1}$)	n	$\bar{x} \pm SD$ (mm^2)
0	12	0.900 ± 0.051
5	12	0.899 ± 0.044
10	12	0.789 ± 0.045*
25	12	0.841 ± 0.059*
50	12	0.802 ± 0.054*
100	11	0.800 ± 0.056*

* Significant differences to the controls ($P<0.05$)

Toxicity of BP1100WD to sea bass larvae was tested for the concentration range of 150-1000 $\mu l\ l^{-1}$. It may be seen that BP1100WD toxicity rapidly increased for 96-hour exposure (Fig.8). At the time larvae aged 5 days. Sea bass larvae resorb the yolk sac at 15.5 °C for five days. Yolk sac resorption is taken as a transition to postlarval stages (Katavic, 1984).

Sea bass postlarval stages showed considerably greater susceptibility to BP1100WD than larval stages. The large slope of the relationship line between medium lethal concentrations of BP1100WD and exposure time shows that the threshold of lethal concentrations is not reached for postlarvae within 96-hours (Fig.8).

Five month old juvenile sea bass proved to be much more resistant to BP1100WD than earlier stages (Fig.8). The slight slope of the line LC_{50} to the time is indicative of the fact that prolonged exposure did not significantly affect mortality. Marked haemorrhages were observed in dead specimens of juvenile sea bass.

4. DISCUSSION

Acute toxicity tests of the oil dispersants BP1100X, BP1100WD, MXG-2 and M-2 proved that they are highly toxic substances to juvenile Liza saliens. BP1100X and MXG-2 showed similar toxicity characteristics.

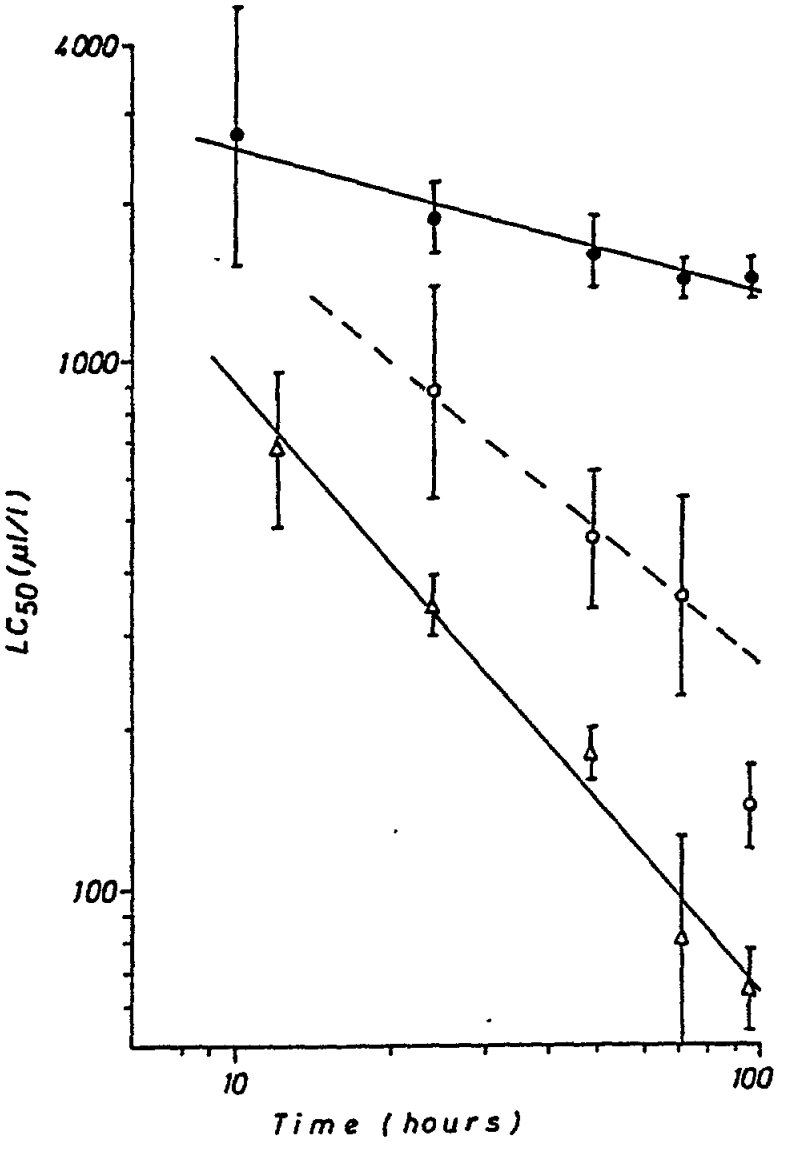

Fig.8 Relationship between medium lethal concentrations (LC_{50}) of BP1100WD and exposure time for larval (o), postlarval (Δ) and juvenile (•) sea bass (Dicentrarchus labrax) stages. Vertical lines mark 95% confidence limits

It may be assumed that the toxic effects of these oil dispersants are primarily manifested on fish gills. Observations of fish behaviour in the tanks showed that they were breathing with difficulty. This fish behaviour in MXG-2 and M-2 dispersants may also be due to the lack of dissolved oxygen in the tanks. A subcutaneous bleeding was also noticed.

In the case of the M-2 dispersant the loss of one of its components is likely to be responsible for the changes of the physical and chemical properties of dispersants, which further increases their toxicity.

For juvenile sea bass, the difference in BP1100WD toxicity between shorter and longer exposures is not considerably marked. This is indicative of the fact that it is the surfactant fraction that is mainly responsible for the toxicity of BP1100WD. It is well known that surfactants are not easily evaporated and not rapidly biodegraded. Therefore, the toxicity of dispersant decreases with the passage of time due to solvent evaporation (Portmann and Connor, 1968). Very marked haemorrhages in dead sea bass individuals are also indicative of the fact that surfactants very strongly affect the gill epithelium (Swedmark et al., 1971; Nagell et al., 1974). Nuwayhid et al., (1980) found that 1000 $\mu l\ l^{-1}$ concentration of BP1100WD caused the lesions of the gill epithelium in Petella vulgata after 24-hours exposure. Swedmark et al. (1971) found narcotic activity and slow decrease of toxicity for non-ionic surfactants.

Results have shown that the toxicity of BP1100WD is reduced, being highest to postlarvae, somewhat less high to larvae and lowest to juveniles. Generally speaking, sea bass eggs show relatively high resistance to BP1100WD effects. Mortality is somewhat more marked at blastula phase and at the time of embryo formation. However, the incubation of sea bass eggs under ambiental conditions shows increased mortality in the morula phase, in the phase of embryo formation after the enclosure of the blastodermal cap and finally just before hatching (Katavic, 1984). This is indicative of the general susceptibility of eggs in these phases of embryonic development. A similar phenomenon was established in herring (Aldredice and Velsen, 1971). High concentrations of BP1002 produce mutagenic effects on the embryos for herring and plaice (Wilson, 1976). The same author found that many eggs had died before blastulation occurred. In view of the fact that the mortality of sea bass embryos in the controls just before hatching was almost equal to that in BP1100WD and in the controls, it is clear that the dispersant has no significant effect on the mortality in this developmental phase.

The beginning of hatching as affected by different BP1100WD concentrations shows no considerable differences compared with controls. The only delay of the beginning of hatching was observed at 1000 $\mu l\ l^{-1}$ concentration. Wilson (1976) found that BP1002 and Finasol ESK prolonged the beginning of hatching of herring and plaice. BP1100WD seems to affect the reduction in the time to 50% sea bass population hatch. Kinne and Rosenthal (1967) considered that stress induced by oxygen deficiency might have caused on premature hatching. By contrast, Corexit extends the mean hatching time in plaice (Wilson, 1976). This is attributed to a bactericidal effect of this dispersant.

BP1100WD in concentrations exceeding 50 $\mu l\ l^{-1}$ caused distortions in hatched larvae manifested as spinal deformities. Deformities of larval axis affected by oil dispersants were recorded earlier as well (Wilson, 1972; 1976). BP1100WD in concentrations of 10 $\mu l\ l^{-1}$ inhibits skeleton formation and ectoderm changes in pluteus phase of the embryonic development of sea urchin (Lönning, 1977).

BP1100WD in higher concentrations shows narcotic effects on sea bass larvae. This was also recorded earlier for oil dispersants (Gyllenberg and Lundqvist, 1976; Wilson, 1976; Kiceniuk et al., 1978).

Statistically significant increased rate of yolk sac resorption compared with the controls was recorded in larvae exposed to BP1100WD concentrations not exceeding 100 $\mu l\ l^{-1}$. This may be accounted for by increased energetic requirements of larvae which probably compensate for the stress induced by a dispersant. Benzene effects on yolk sac resorption of herring larvae was explained similarly (Struhsaker et al., 1974).

The rapid increase of acute toxicity of BP1100WD to sea bass larvae between 72 to 96 hour exposure may be attributed to the transition of larvae to postlarvae. Namely, complete yolk sac resorption in sea bass larvae takes 5.5 days at 15.5 °C (Katavic, 1984). Larvae used in our experiment were approximately of the same age. The phenomenon of increased mortality of larvae in the phase of reduced yolk sac content is generally well known (Farris, 1960; Kuo et at., 1973; Sanders, 1975; Katavic, 1984). That is, the dispersant only potentiates larval mortality in this critical phase.

More marked susceptibility to BP1100WD of postlarvae than that of larvae may be partly explained in terms of the casing of endogenic nutritive source.

It seems that marine organisms "in situ" will probably have the ability of avoidance of oil dispersants (Portmann, 1972; Gyllenberg and Lundqvist, 1976). Thus probably the problem of toxicity in the natural environment is mainly connected with immobile and poorly mobile forms like eggs and larvae. Sea bass spawn in nature from December to March (Katavic, 1984) when the sea water temperature is 12-13 °C. Nagell et al.(1984) found very high temperature coefficient values (Q_{10}) for oil dispersant toxicity. Accordingly, oil dispersant toxicity may depend on temperature which probably is an additional factor of toxicity reduction for eggs and larvae of sea bass in a natural environment.

5. CONCLUSIONS

BP1100X and BP1100WD are highly toxic to juvenile Liza saliens. Their acute toxicity was much higher to juvenile Liza saliens than to some fish species of the northern seas. These differences in toxicity may be due either to the higher testing temperature (20 °C) or to greater susceptibility of Liza saliens to these dispersants.

MXG-2 and M-2 dispersants seem to biodegrade quickly and affect dissolved oxygen levels both in aerated and non-aerated tanks. Therefore, toxicity tests of these substances under static conditions were rather difficult apart from the fact that fish were much more susceptible.

MXG-2 and M-2 are highly toxic to juvenile Liza saliens so that, even concentrations lower than 100 $\mu l\ l^{-1}$ and 1000 $\mu l\ l^{-1}$ will be lethal at 96-hour exposure of fish.

Fertilized sea bass eggs are relatively resistant to the effects of BP1100WD for the first 24-hour exposure. Very low mortality increases successively with higher dispersant concentrations. A delay in the beginning of hatching was recorded at the BP1100WD concentration of 1000 $\mu l\ l^{-1}$. The larvae hatched after exposure to concentrations of 100 $\mu l\ l^{-1}$ and above showed spinal deformities.

Larvae probably have an endogenic energy source which allows the compensation of stress caused by dispersants. This is evident by a higher yolk sac resorptional rate of sea bass larvae. Therefore acute toxicity of BP1100WD shows an increase for juvenile larval and postlarval stages of Dicentrarchus labrax.

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THE IMPORTANCE OF TEMPERATURE AND LIGHT CONDITIONS ON THE TOXICITY OF OIL, OIL DISPERSANT AND OIL/DISPERSANT MIXTURE TO Artemia salina AND METABOLIC RESPONSES OF Artemia salina TO OIL AND OIL/DISPERSANT MIXTURE

by

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1. INTRODUCTION

Crude oil contains thousands of different hydrocarbons and other compounds. Oils from different fields may differ widely in physical, chemical and thus toxicological properties. Apart from the type of oil other factors markedly influence the toxicity of crude oils to marine organisms: state of oil (thin oil films, thick oil films, water-in-oil emulsions, oil-in-water emulsions) climatic and weather conditions (wind and waves, temperature and sunlight). Finally the toxicity of oil may be altered throughout interactions with other compounds such as dispersants used in cleaning-up processes and other chemicals such as phenol, sulphides, ammonium compounds, etc. Oil represents an obvious biological hazard in the coastal environment and studies of its impact on marine organisms are greatly needed.

Solvent based dispersants are among the chief ways of removing oil from shores. Although recently new dispersants have been developed (usually by replacement of the highly toxic aromatic hydrocarbons of their solvent part by aliphatic hydrocarbons) which are much less toxic than the first ones, dispersants still are toxic substances. As for oils the harmful effects of dispersants on aquatic life may be altered by the influence of environmental factors and the presence of other compounds acting in the environment, especially oil.

In this paper we have studied the toxicity of an oil (Tunisian crude oil zarzaitine type), an oil dispersant (Finasol OSR2) when acting alone and when acting together, to the brine shrimp Artemia salina. In order to evaluate the influence of two environmental conditions (temperature and light) on the three toxicants (oil, dispersant, oil/dispersant mixture) all toxicity tests were run under two different temperatures and three different light conditions. Aiming to investigate if larval stages of Artemia are more sensitive than adults to oil and dispersant and also if the responses of young individuals are the same with those of adults when exposed to toxicants under different light conditions, we have repeated the toxicity experiments using larvae of Artemia.

Insight into the impact of a pollutant on marine or estuarine organisms may be gained from two types of biological and chemical investigations:

(a) short-term acute toxicity tests;
(b) organism-environment transfer studies;
(c) physiological studies; and
(d) field studies (Anderson et al., 1974).

In the case of oil pollution, field studies have limited value for the estimation of the oil impact on natural communities since results are often confounded with the effects of detergents used in clean-up processes. Both types of laboratory studies (acute toxicity and sublethal toxicity tests) support valuable information for the toxicity of oils and oil dispersants. Since in the case of oil pollution treatment, oils and detergents are acting in combination, a realistic approach of laboratory studies must also include the combined action of these substances on marine organisms. Although acute toxicity studies which measure the mortality due toa toxicant are very useful for the determination of the range of animal tolerance, other effects causing physiological alterations may be detrimental to a population's survival.

This paper concerns also research on the effects of an oil, an oil dispersant and of the mixture of the oil and dispersant to a physiological process, the respiration of the brine shrimp Artemia salina.

2. MATERIAL AND METHODS

Artemia salina hatched from commercially available cysts was used as test animal: adults in all types of experiments but also larvae (36 ± 12 hours old) for some experiments. The acute toxicity was estimated by determining the LC_{50} 48 h (concentration of a toxicant which kills the 50% of the test animals after 48 hours exposure) according to the Bliss (1938) method. All experiments (static bioassays) were run in constant temperature rooms at 14 ± 0.5 °C and 22 ± 0.5 °C. Adults of Artemia were put individually in 30 ml glass jars sealed with Teflon-lined caps. Larvae were put in groups of about 30 in 200 ml Erlenmeyer flasks. Oil-water mixtures for use in bioassays were prepared from oil-in-water dispersions (OWDs). OWDs were prepared by adding measured volumes of oil (Tunisian crude oil zarzaitine type) to artificial sea water (prepared by mixing distilled water with instant ocean synthetic sea salts) and shaking the mixture vigorously for 15 minutes at approximately 2000 cycles min^{-1} on a shaker. Detailed characteristics of the tested OWDs and the resulting concentrations of hydrocarbons at various times could not be obtained. The dispersant solutions were prepared by diluting a stock solution of 80 ppm Finasol. The oil/dispersant mixture contained equal parts of oil and Finasol. In no one case were the test mixtures aerated during the experiments.

The range of experimental concentrations was fixed by preliminary tests. Generally 6-7 concentrations of the test mixture plus a control were used in each bioassay. A minimum of 30 animals were exposed at each concentration in all cases. For the adults of Artemia the acute toxicity (LC_{50} 48 h) was estimated at two temperatures: 14 and 22 °C and for the larval stages (36 ± 12 hours old) at 22 °C. For both adults and larvae the bioassays were performed under three light conditions (continuous light, continuous dark and photoperiod (12 h light, 12 h dark). The light intensity ranged from 220 to 300 lux. At 24 and 48 hours the containers were examined, mortalities were recorded and at 24 h dead individuals removed. Test animals were put in the containers immediately after the preparation of the mixture because as it was previously found (Verriopoulos and Moraitou-Apostolopoulou, 1983) for all three tested mixtures a significant decrease of their toxicity is noticed with time.

The difference between the linear regressions by which the LC_{50} under various experimental conditions were determined, were tested statistically by the t-test (statistically significant between 95 and 99.99% level).

For the respiration experiments and for oil and Finasol three series of toxicity tests were performed. In the first, the test concentrations were prepared immediately before the experiment (0 h test). In the second, the stock solution was prepared 48 hours before the experiment (48 h test) and in the third 96 hours (96 h test). For the oil/dispersant mixture only a 0 h solution was tested. This was done because in a previous work (Verriopoulos and Moraitou-Apostolopoulou, 1983) we have noticed that the toxicity of oil and especially of Finasol was reduced with time ("age" of stock solution). More precisely the LC_{50} 48 h of oil for Artemia was 297.89 ppm when the stock solution was prepared immediately before the experiment (0 h solution), 530.0 when the stock solution was prepared 48 hours before the experiment (48 h solution) and 407.96 when prepared 96 hours in advance. The decrease of toxicity of Finasol was significant and varied as follows: LC_{50} 48h: 0 h solution: 0.939; 48 h solution: 10.058; 96 h solution: 21.094. As various parameters such as size, sex, physiological conditions, life history etc., may influence the respiration of the test animals, we have performed every experiment with four groups of animals: (a) large males (8-10 mm length), (b) medium-sized males (6-8 mm), (c) large females (8-10 mm) and (d) medium-sized females (6-8 mm). In addition, all animals used in any experiment (experimental and controls) came from the same source and were hatched and reared under the same conditions.

Respiration rates were determined potentiometrically with a Radiometer PHM 73 Blood Gas Analyser, using 20 ml disposable syringes as respiration chambers. The analyser chamber was connected to a Haalte constant temperature circulator maintained at 22 ^{o}C throughout. Artemia were placed in syringes filled with test water. The syringes were carefully capped to exclude all air bubbles, incubated upside down in a darkened constant temperature room and tested 3-5 hours later. Syringes were removed from the constant temperature room, inverted to disrupt possible oxygen gradients, and aliquots were injected directly into the Radiometer analyser chamber. Controls without Artemia exhibited negligible changes in oxygen concentration. The Radiometer electrode was calibrated prior to the first measurement. Reading in mm Hg were converted to percent saturation (using the oxygen solubility tables of Truesdale and Downing (1954). Respiration rates in μl O_2 were calculated directly from the change in percent saturation before and after the experimental period.

3. RESULTS

Table I gives the LC_{50} 48 h of the three toxicants calculated according to the Bliss method.

Oil presented a low toxicity to Artemia. On the contrary Finasol proved very toxic. The mixture oil/Finasol exhibited an intermediate toxicity, its harmful effects being obviously due to the dispersant's presence. Temperature seems an important factor of the level of toxicity of the three tested solutions to Artemia. When acting under the same light conditions all solutions were much more toxic at the higher tested temperature (22 ^{o}C). All differences between the calculated LC_{50} 48 h values were statistically significant with one exception: When Finasol is acting under continuous dark conditions the LC_{50} at 14 and 22 ^{o}C are not statistically different.

Light conditions also seem to influence the toxicity of the three toxicants to Artemia. However, only in the case of oil when acting under the same temperature but under different light conditions the differences between the calculated LC_{50} were in all cases statistically significant. Oil exerted its highest toxicity to Artemia when acting under continuous dark conditions. A statistically significant increase of its toxicity was noted under continuous light and especially under photoperiod conditions. The influence of light conditions on the toxicity of Finasol and Finasol/oil mixture was the same. Contrary to what was found for oil, both solutions demonstrated more toxicity when they are acting in the dark. Under light conditions the two solutions were less toxic and a further decrease of their toxicity was noticed under photoperiod. However, the differences between the LC_{50} values when the two solutions are acting at light and at dark were significant only in one case (out of 4): for the mixture of Finasol/oil at 22 °C. More pronounced was the decrease of toxicity from light to photoperiod conditions: only in one (out of 4) cases the difference between the calculated LC_{50} values was not significant: in the case of Finasol/oil acting at 22 °C.

Table I

LC_{50} 48 h (ppm) of the three toxic solutions for Artemia at various temperature and light conditions

Light	Continuous dark			Photoperiod			Continuous light		
temperature	14 °C	22 °C		14 °C	22 °C		14 °C	22 °C	
stage	Adults	Adults	Larvae	Adults	Adults	Larvae	Adults	Adults	Larvae
Finasol	1.83	0.83	2.72	8.001	1.19	3.427	2.42	1	2.95
Oil/Finasol	34.446	4.24	5.85	137.21	8.2	7.99	62.5	6.04	7.66
Oil	11.865	677.27	260.84	7309.8	464	201.4	9982.8	616.74	250.84

Fig. 1 illustrates the impact of temperature and light conditions to the LC_{50} of oil to Artemia, Fig. 2 that of Finasol and Fig. 3 that of Finasol/oil mixture. The estimation of multiple linear regression analysis is:

for oil, $z = 26.187.66 - 1141.63x - 40.48y$ (Coefficient of Determination $R^2 = 0.9295$)

Finasol, $z = 9.2833 - 0.385x + 0.0158y$ (Coefficient of Determination $R^2 = 0.3835$)

Finasol/oil, $z = 196.406 - 8.987x + 0.621y$ (Coefficient of Determination $R^2 = 0.5950$)

where z = concentration of the toxic solution (in ppm)
y = light (duration of light in hours)
x = temperature (°C)

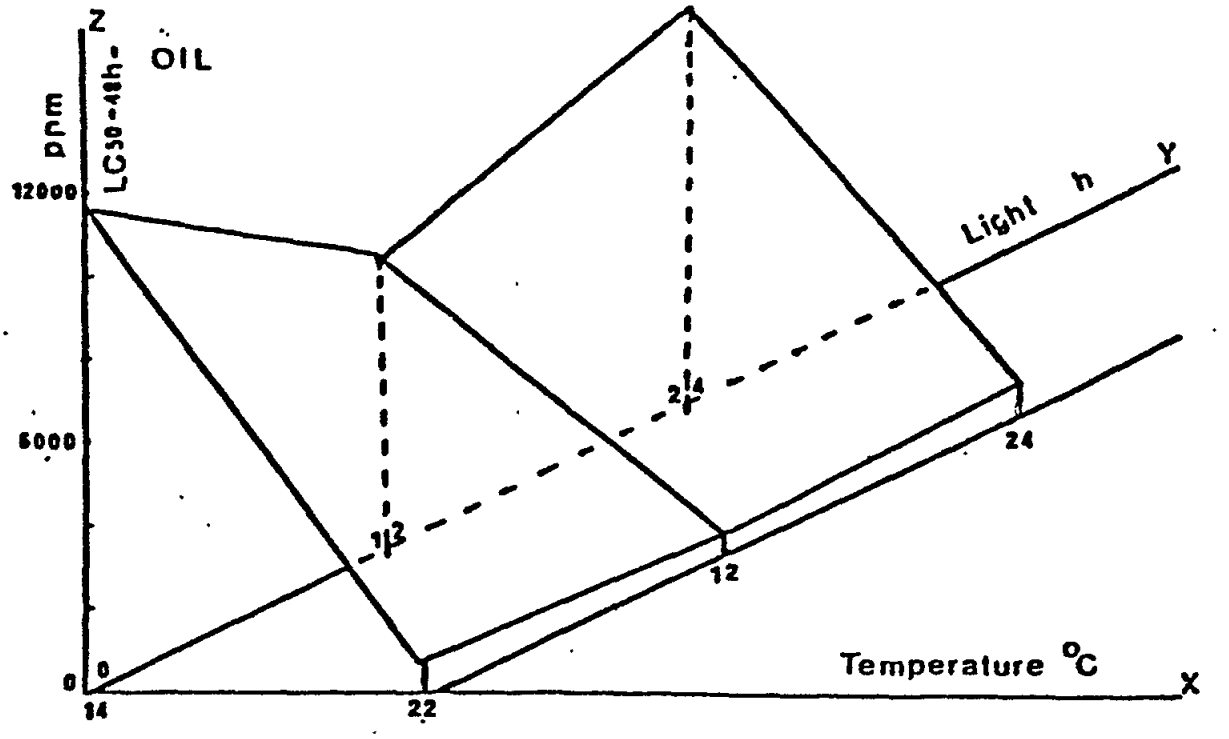

Fig. 1 Impact of temperature and light conditions to the LC_{50} 48 h of oil (Tunisian crude oil zarzaitine type) to Artemia

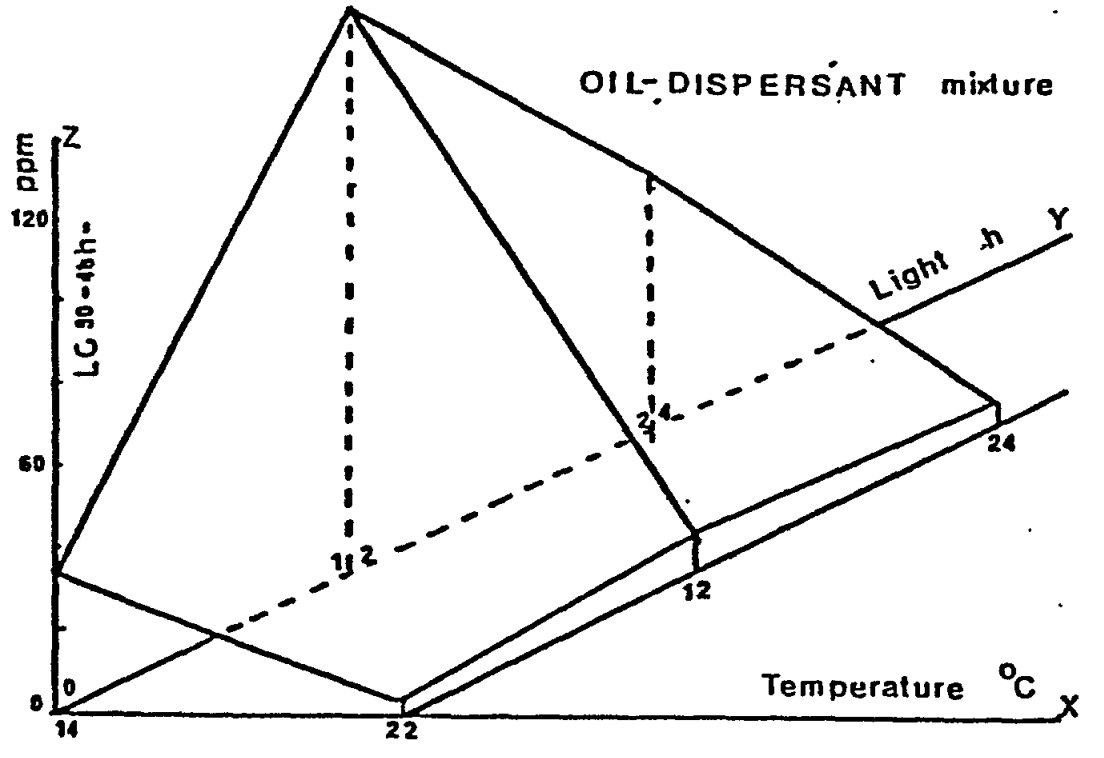

Fig. 2 Impact of temperature and light conditions to the LC_{50} 48 h of oil dispersant (Finasol OSR2) to Artemia

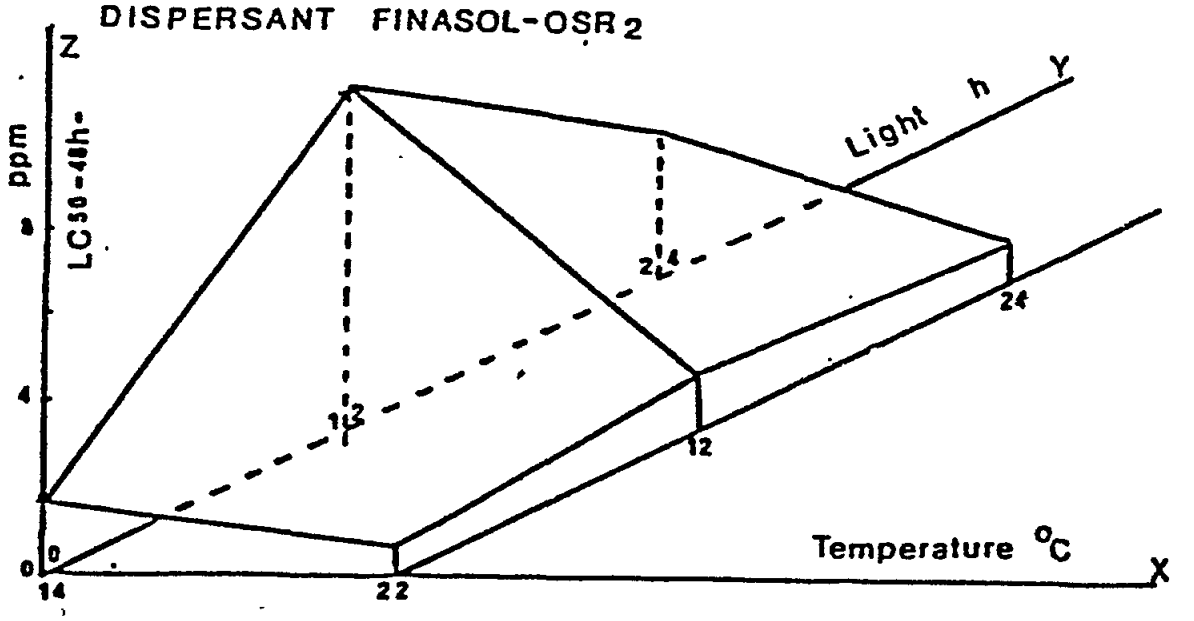

Fig. 3 Impact of temperature and light conditions to the LC_{50} 48 h of oil/dispersant mixture to Artemia

Larval stages of Artemia proved more sensitive to the three toxicants, under the same temperature and photic conditions, than adults. All differences between the LC_{50} of adults and larvae were statistically significant. Furthermore light conditions influence the toxicity of the three toxic solutions to the larvae in the same way as for adults: non-significant decrease of their toxicity at light.

Table II shows the measured respiration rates of Artemia. Results include the rate on non-exposed (control) animals and that of exposed to oil, Finasol and oil/Finasol mixture.

As for the reasons mentioned above, the respiration rates between controls of the various experiments present differences and in order to render easier the comparison of the results, we have converted the measured values of respiration rates as percentages of the control values (Fig. 3).

Although results of various experiments show differences, it is clear that all three toxic solutions elicit changes in respiratory rates of Artemia. The direction (stimulation or suppresssion) of the change is the same for the three solutions and seems concentration dependent. There is a general trend of decrease of the respiration rate at the lower and up to LC_{50} 48 h concentrations. At higher concentrations a usually important stimulation of respiration rate was observed. Finally at the highest tested concentrations the respiration undergoes an important suppression. Surprisingly, in most cases an important respiratory change was noticed in very low toxicant concentrations (1/40 to 1/15 of the LC_{50} 48 h). The direction of this respiratory change (suppression or stimulation) was not always the same as that observed at higher and up to LC_{50} 48 h concentrations.

Table II

Respiration rates and standard deviation ($\mu l\ O_2\ animal^{-1}\ hour^{-1}$) of non-exposed (controls) and exposed animals

Concentration (ppm)	males (8-10 mm)	males (6-8 mm)	females (8-10 mm)	females (6-8 mm)
		FINASOL (0 h solution)		
0	90.49 ± 1.58	72.16 ± 2.65	93.07 ± 2.57	72.26 ± 3.16
0.5	87.68 ± 3.7	66.34 ± 8.66	87.13 ± 5.8	50.77 ± 4.16
1	53.09 ± 13.86	-	-	-
20	94.51 ± 12.32	70.42 ± 11.34	92.66 ± 2.75	63.02 ± 6.89
30	36.32 ± 9.17	36.22 ± 9.76	-	35.18 ± 0.51
		FINASOL (48 h solution)		
0	65.57 ± 2.98	38.72 ± 3.42	56.75 ± 5.71	42.75 ± 1.98
0.5	47.83 ± 6.61	35.66 ± 1.65	51.74 ± 2.4	33.03 ± 2.14
10	47.77 ± 9.1	25.27 ± 4.83	-	-
20	75.04 ± 16.6	42.65 ± 7.29	-	-
30	61.43 ± 6.14	37.0 ± 7.5	-	21.9 ± 10.4
		FINASOL (96 h solution)		
0	66.59 ± 6.23	35.17 ± 4.32	70.57 ± 6.36	29.4 ± 1.72
0.5	-	42.11 ± 2.31	74.8 ± 10.3	33.52 ± 9.6
20	-	41.68 ± 11.5	-	29.05 ± 8.2
21	52.06 ± 6.89	33.66 ± 3.32	52.24 ± 3.11	32.75 ± 2.94
30	93.67 ± 2.35	59.05 ± 4.69	-	62.92 ± 3.62
		OIL (0 h solution)		
0	39.85 ± 5.61	23.25 ± 4.16	61.87 ± 13.7	24.73 ± 0.54
20	64.05 ± 1.64	-	66.02 ± 11.8	30.65 ± 3.82
300	-	35.37 ± 4.15	49.51 ± 9.08	28.45 ± 2.15
500	-	49.65 ± 6.93	-	42.53 ± 4.51
		OIL (48 h solution)		
0	62.44 ± 6.69	40.92 ± 5.0	53.14 ± 4.48	28.41 ± 2.13
20	-	14.49 ± 0.35	-	17.63 ± 1.33
500	-	22.41 ± 6.57	76.83 ± 7.63	37.94 ± 3.58
800	69.33 ± 15.6	18.96 ± 3.18	-	23.8 ± 0.03
		OIL (96 h solution)		
0	48.33 ± 3.71	28.39 ± 1.91	65.42 ± 4.98	17.71 ± 6.70
20	47.66 ± 8.8	21.77 ± 6.78	47.91 ± 6.15	24.59 ± 4.81
410	42.31 ± 7.13	18.5 ± 4.00	56.57 ± 19.8	-
500	60.65 ± 6.31	35.47 ± 5.78	-	29.43 ± 7.65
800	-	-	-	16.81 ± 1.56
		OIL + FINASOL (0 h solution)		
0	66.85 ± 16.4	45.1 ± 6.21	81.56 ± 4.55	40.92 ± 4.35
1	70.62 ± 19.51	35.83 ± 6.78	56.85 ± 16.6	34.04 ± 3.88
5.5	55.02 ± 12.8	33.2 ± 5.06	69.28 ± 9.4	44.35 ± 11.3
50	38.99 ± 4.39	22.38 ± 4.74	50.56 ± 1.7	21.44 ± 2.9

The magnitude of the observed respiration change presents differences not only between the three toxic solutions but also between the various "ages" of solutions of one toxicant and even between the various groups of experimental animals. Although non-significant differences have been established (by the t-test) between males and females, differences have been found between the two size classes of Artemia. Both males and females of the large size class were proved more sensitive to toxicant stress and presented more important respiratory changes. This is clearly demonstrated in Fig. 4 where the respiratory rates of the two size classes (male and females put together) of Artemia after exposure to Finasol is shown.

4. DISCUSSION

The results of short-term acute toxicity tests indicate that oil exerts low toxicity to Artemia. Results on oil toxicity present generally wide differences. A number of factors may influence this variability: type of crude oil (Kauss et al., 1973; Kühnhold, 1974; Anderson et al., 1974; Templeton et al., 1975), period of time between the mixing of oil and water and the addition of animals to the mixture (Verriopoulos and Moraitou-Apostolopoulou, 1983).

Although an analysis of the hydrocarbon content of the test solutions was not possible, data from the literature show that a small fraction of the oil added as dispersed fine droplets is measured as oil-in-water phase. According to Anderson et al. (1974) for the Kuwait crude oil when 100 ppm were added to the test solution, the total hydrocarbons (as determined by IR) in the aqueous phase of the oil-in-water dispersion was 20 ppm (for 1000, it was 27 and for 10000, 36). Unlike the water soluble fractions (WSF) of oils which are richer in the more soluble aromatic hydrocarbons, the OWDs are expected to resemble that of the parent oil since most of the hydrocarbons are present in dispersed droplets.

The dispersant Finasol OSR2 proved very toxic to Artemia. The theory behind the use of dispersants in oil incidents is that they accelerate the rate of natural degradation of the oil. The acceleration of the biological degradation is achieved by increasing the surface area of oil by dispersion. However, the dispersant may make the oil more readily available to organisms with a particular structure of life style. The organisms at high risk are probably the feeders like Artemia which extract small suspended particles from the water. According to Cowell (1976) the hydrocarbon solvents of dispersants are liable to penetrate into plants through the lypophilic surface and penetration is a crucial adjunct of toxicity. Once inside the plants the detergents may dissolve cell membranes and cause loss of cell sap. Apart from their evident direct toxicity to organisms, dispersants exhibit a high demand for oxygen (500,000 to 1,000,000 mg l^{-1}) resulting, when used in closed bays with limited circulation, in a significant lowering or depletion of dissolved oxygen. Similar results on the toxicity of oil, dispersant and oil/dispersant mixture have been reported and discussed in detail in a previous paper (Verriopoulos and Moraitou-Apostolopoulou, 1983).

An important and in all cases significant increase in toxicity of the three tested toxic solutions was noticed with an increase in temperature from 14 to 22 °C. Temperature changes influence both toxicants and organisms. Changes in temperature influence the transport of oil in water changing its viscosity, its solubility and the dispersant components as well as the

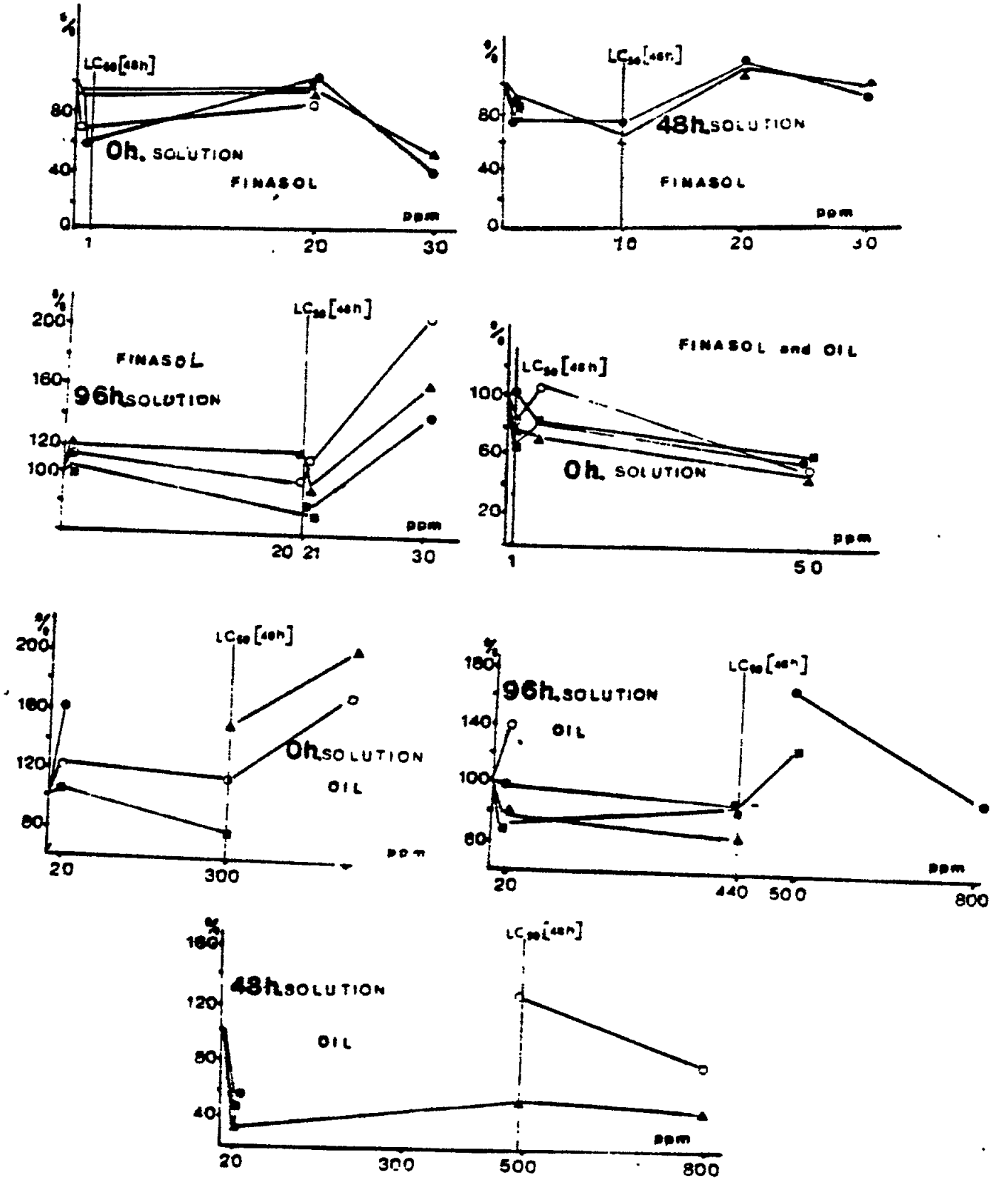

Fig.4 Respiratory rates (% of the controls) of Artemia salina exposed to oil, Finasol, and to oil/Finasol solutions of various "ages". o = large females (8-10 mm); = large males (8-10 mm); = medium sized males (6-8mm); = medium sized females (6-8 mm)

stability of an emulsified or suspended oil. As temperature increases the viscosity of the oil decreases and the solubility of the non-volatile components, which remain longer than the volatiles in the test solution, increases. The elevation of temperature causes, within the tolerance range of an organism, a metabolic rate increase and also numerous biochemical and physiological changes. Temperature also changes the permeability of cellular membranes.

Similar results, i.e., increase of pollutants' toxicity with temperature elevation, have been referred to by other authors (Eisler, 1971; Moraitou-Apostolopoulou et al., 1979). The observed pronounced increase of the sensitivity of Artemia to oil and Finasol with the elevation of temperature from 14 to 22 °C is of importance in temperate regions where animals encounter similar temperatures during summer. The impact of light conditions on the toxicity of oil mainly but also, to a lesser degree, of Finasol and Finasol/oil mixture seems very important. The increase under continuous light and photoperiod conditions must be attributed to the direct effect of light both on oil and Artemia. There is evidence that polar hydrocarbon derivatives are generated from oil by photo-oxidation (Lysyj and Russel, 1974). These polar hydrocarbons tend to dissolve into solution from an oil slick which raises the total concentration of oil-derived hydrocarbons with time. Exposure of petroleum constituents to oxygen and light results in their oxidation. This oxidation usually produces more soluble compounds and/or toxic acid. For example the oxidation of n-octanol (solubility 1 ppm) yields n-octanic acid (solubility 600 ppm). The susceptibility of animals to oil dispersants has been found to vary over dark and light periods in the case of animals such as the limpet Patella vulgata which demonstrate circadian rhythmus (Dicks, 1975). Light also directly influences the physiology of marine organisms e.g. by increasing their respiratory rates rendering thus oil more available to animals. In the planktonic copepod Calanus finmarchicus exposure to light results in a sharp increase in its respiratory rate (Marshall et al., 1935). The decapod crustacean Hemigrapsus oregonesis showed a higher oxygen consumption at light than crabs kept under constant darkness (Dehnel, 1955).

Finally there is evidence that light may activate harmful effects of oil. Cusachs and Steele (1969) have suggested that carcinogenesis by polycyclic aromatics may result from sublethal photodynamic effects. These authors have proposed the involvement of singlet oxygen in polycyclic aromatics carcinogenesis. Khan and Kasna (1976) have proposed an optical residue singlet oxygen theory of photocarcinogeneticity in which the polycyclic aromatics are first bound to the cellular constituent. The resulting residual molecule could continue to absorb light from the environment and sensitize the formation of singlet oxygen. The observed tendency in the decrease of dispersant toxicity to Artemia when acting at light must probably be attributed to the photooxidation of some part of its solvent and/or surfactant constituent which becomes less active, less soluble or more volatile.

The oil/dispersant mixture demonstrated the same reaction as the dispersant to photic conditions because its toxicity is mainly due to the dispersant fraction. A further decrease of dispersant mixture compared with their toxicity at light, was observed when the two solutions are acting under photoperiod conditions. As photoperiod in our experiments began with 12 hours light, it may be assumed that light during this period has exerted its "beneficial" effect on dispersant and oil/dispersant toxicity. During the

following dark period Artemia individuals have probably demonstrated the reaction at dark referred to in the bibliography, that is, lower respiratory and filtration rates. In this way, the experimental animals have "received" smaller amounts of pollutants and thus proved more resistant. The observed increase of oil toxicity under photoperiod conditions compared with their toxicity at light seems difficult to explain.

The respiratory chain is of vital significance for supplying energy to living cells. Respiration represents an important physiological index of an organism because respiratory rates reflect the metabolism of an animal and its overall functional well-being.

The electron transport along the respiratory chain may be inhibited at specific sites by different factors. Physiological stresses causing respiratory responses induced by change of environmental parameters such as temperature, salinity, dissolved oxygen, but also chemical pollutants, have been well documented in the literature. As respiration is sensitive to a whole array of environmental as well as biological variables, respiratory rate was used as a measure of sublethal stress. Exposure to oil and to oil dispersant may also constitute a physiological stress to marine animals. If this is so, Artemia might be expected to show a respiratory response to oil, oil dispersant and also to oil and dispersant mixture. Our results have demonstrated that all three toxic solutions cause physiological changes to the respiration of Artemia.

Significant respiratory responses have been found to occur at high nominal oil concentrations. Similarly high values of LC_{50} 48 h of oil to Artemia have been noticed recently (Verriopoulos and Moraitou-Apostolopoulou, 1983). This is due to the fact that the aqueous phase of OWDs usually contains one to two orders of magnitude less than the amount originally added. Furthermore a rapid decline in the concentration of oil hydrocarbons in the aqueous phase is observed. As a result, an important decrease of the toxicity of oil, Finasol and oil/Finasol solutions with time (48 h old solution, 96 h old solution) has been noticed (Verriopoulos and Moraitou-Apostolopoulou, 1983). An analogous decrease in toxicity of the oil and Finasol was also found in our present results.

As sublethal impairment of the capacity of an animal to perform and adapt occurs when important respiratory changes are caused, it can reduce the chance for survival and the potential for growth and reproduction. The constraints of sublethal stress though possibly slight for individual members of a population, may be highly detrimental to populations with consequences as severe as extinction.

The nature of respiratory response (increase or decrease) presents some variability but shows the same pattern for the three solutions and appears to be concentration dependent. More variable and less important respiratory reactions are generally noted at the lower concentrations up to LC_{50} 48 h (with the exception of very low concentrations where in some cases important changes have been noted). At concentrations higher than LC_{50}, an important increase of respiration has usually been observed and could be mainly attributed to the observed high motility of Artemia at these concentrations. Similar activity related to respiratory increases has been referred to by Zeuthen (1955) and Anderson et al. (1974). The observed abrupt lowering of respiration at very high concentrations must be the result of a "moribund" state of animals imminent to death.

The respiratory responses to pollutants, as referred to in the literature, present great variability. The nature of the response (increase or decrease) seems to vary not only between the different toxic substances but also between species and often is a function of the concentration of the toxicant.

As far as heavy metals are concerned, Jones (1942) reported that the respiration rate of Gammarus pulex and Polycelis nigra was raised markedly by copper and mercury as did Hunter (1949) for a marine gammarid exposed to copper. Corner and Sparrow (1956) found these metals to depress the respiration rates of Artemia, while Bernard and Lane (1961) found that increasing copper concentrations caused barnacle cyprids to increase and then decrease their respiratory rates.

Kapoor and Griffiths (1976) have noticed a relatively constant increase of respiration rate for Phasganophora capitata at low copper concentration and an exceptionally large increase at the lethal threshold level of copper. A marine worm Nereis virens (Raymont and Shield, 1962) and the sunfish Lepomis macrochirus (O'Hara, 1971) have shown similar results. Reeve et al. (1977) working with natural plankton populations found little indication of any systematic effect of two metals (copper and mercury) on respiration at concentrations up to and beyond those required to produce a lethal effect on 50% of population in 24 hours. The same authors conclude that respiration cannot be considered as a sensitive indicator of sublethal stress. Consistent changes in respiration rate are usually only indicative of imminent death.

A great variability of data is also noticed concerning the effects of oil and its constituents on marine organisms. Anderson et al. (1974) have found a stimulation of the respiratory rate of Cyprinodon when exposed to the water soluble fractions (WSF) of 2 fuel oils while the WSF of the bunker C oil caused a depression of oxygen consumption of this fish. The same authors working with post-larvae of Penaeus found for the two oils the same pattern of respiratory responses: lowering of the oxygen consumption at lower concentrations and respiratory rates near those of the controls at intermediate WSF concentrations. Struhsaker et al. (1974) found that lower concentrations of benzene (a water soluble component of crude oil) accelerated the metabolic rate of larvae of pacific herring and northern anchovy while at higher concentrations the metabolic rate is delayed. Dunning and Major (1974) found that cold sea water extracts of different oils lowered the respiratory rate of Mytilus edulis.

Considering the bulk of published literature on the effects of toxic agents on the respiration of marine animals one remains impressed by the variability of the results. In many experiments authors also refer to strong variations between the experimental animals of one experiment. Respiration is affected by a great variety of factors including life and nutritional history of animals and stress of handling. Thus an important source of variation is introduced and this becomes particularly important, when working with natural populations. In order to overcome this disturbing factor, in each experiment both controls and experimental animals came from the same batch, hatched simultaneously and were reared under the same conditions. In fact we have noticed important differences in the respiration rate between the controls of the various experiments.

In our results, but also in other works, the nature of respiratory response has been found to be a function of concentration. It is possible that in other works where only a stimulation or a depression of the respiration rate is referred to after exposure to a toxicant, the results concern only the range of the tested concentrations and eventually an inverse respiratory reaction could be observed outside the tested concentration range.

Generally, consistent respiratory changes have been referred to at high concentrations and thus respiration cannot be characterized as a general sensitive pollutant stress. However, in some of our experiments significant respiratory reactions have been noticed at low concentrations. Similarly Anderson et al. (1974) have found the respiratory responses of Penaeus postlarvae when exposed to 2 fuel oil to be more pronounced at lower than intermediate concentrations. In previous works (Moraitou-Apostolopoulou and Verriopoulos,1979, 1982; Moraitou-Apostolopoulou et al., 1979) we have noticed a significant increase of the respiratory rate of Acartia clausi after exposure to sublethal concentrations of three heavy metals (copper, cadmium, chromium). Valuable information could therefore be gained from detailed research on the effects of low concentrations.

On the other hand, respiration is relatively easy to monitor in live animals and, more important, tests are rapid. Respiration therefore could be used at least in urgent screening tests.

Finally, an interesting observation of our results is that large animals seem more sensitive to respiration changes than smaller ones. Cox and Anderson (1973) have also noticed that the sensitivity of the shrimp Penaeus increases as their size increases. Similarly Anderson et al. (1974) have noticed that the changes in respiratory rate of large individuals of Palaemonetes pugio was more pronounced than that of smaller animals.

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EFFECTS OF PRE-EXPOSURE ON THE TOLERANCE OF *Artemia salina* TO OIL AND OIL DISPERSANT

by

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1. INTRODUCTION

The effects of oil pollution on the marine environment have received increasing attention over the last two decades. In the open sea, concentrations of hydrocarbons are generally low. By contrast, in areas affected by massive contamination the hydrocarbons may be present in high concentrations.

Chronic low level contamination of coastal waters originating primarily from the discharge of oil from near shore ship operations, via urban and industrial sewage effluents etc. often occurs. In these cases no mass mortalities are observed and the impact of this kind of pollution has not yet been quite clarified. Mackin and Hopkins (1961) have postulated that in some coastal areas the continuous input of petroleum has little or no demonstrable adverse effect on populations or productivity, while other investigators have suggested that biological processes of estuarine organisms can be effected by low levels of petroleum (Gilfillan, 1973; Jacobson and Boylan, 1973). Dunning and Major (1974) concluded that the sum of innumerable small leaks and drops probably constitutes the major long range environmental problem.

The mechanisms possessed by organisms for handling oil pollution assume particular importance under contaminated conditions. Under these conditions the ecological balance may be tilted as the more tolerant organisms are favoured.

In the case of metal pollution metal resistant strains of organisms have been referred. The tolerant organisms have been collected from contaminated areas (Russell and Morris, 1970; Whitton, 1970; Bryan and Hummerstone, 1971, 1973a,b; Brown, 1976; Moraitou-Apostolopoulou, 1978; Moraitou-Apostolopoulou and Verriopoulos, 1979; Moraitou-Apostolopoulou *et al.*, 1979a,b).

For various animals it has been demonstrated that increased metal tolerance can be obtained under laboratory conditions after pre-exposure to low concentrations of metals (Saliba and Ahsanullah, 1973; Sinley *et al.*, 1974; Saliba and Krzyz, 1976; Moraitou-Apostolopoulou *et al.*, 1983).

The aim of this paper was to try to find out if there is a possibility of induction of increased tolerance to oil and oil dispersant in marine organisms after pre-exposure to these pollutants as it occurs in nature under chronic oil pollution. In this way we should be able to better understand the importance of chronic exposure of marine organisms to oil pollution.

2. MATERIAL AND METHODS

Adults of Artemia hatched from commercially available cysts (New Technology Artemia salina Revolution) were used as test animals. The hatching of the cysts and the rearing of Artemia was performed in constant temperature rooms at 22 ± 0.50 °C in synthetic sea water (synthetic type).

The four toxic solutions tested were: crude oil (Tunisian crude oil zarzaitine type), two oil dispersants (Finasol OSR2, Finasol OSR5) and their mixture. The type of oil/water mixture tested was oil-water dispersion (OWD). OWDs have a hydrocarbon composition very closely resembling that of the parent oil (Anderson el al., 1974). OWDs and Finasol solutions were prepared by diluting a known amount of stock solution of the toxicant to one litre of synthetic sea water. After the addition of the toxicant, the stock solutions and the test concentrations were shaken at approximately 1.000 cycles per minute for 30 minutes for the oil and 15 minutes for Finasol. Detailed characteristics of the tested OWDs and the resulting concentrations of hydrocarbons could not be obtained. The mixture of oil and dispersant contained equal parts of oil and Finasol.

Two age groups of Artemia were used in the experiments: group A(4-5 mm long), group B(6-7 mm). In some experiments (exp. 1 to 3a see Table I) Artemia were fed during the experiments. As food, Germalyne, a dietetic human nutricent, derived exclusively from wheat was used. Artemia were exposed for various periods of time to a standard concentration of one of the four toxic solutions (pre-exposure acclimation period). Thereafter their tolerance to this toxic solution was tested. For some experimental groups, after the pre-exposure period, animals were transferred to clean sea water for some period of time (detoxification period). After the detoxification period the tolerance of animals to the pre-exposure toxicant was tested. The whole plan of experiments is shown in Table I.

Two indices of toxicity were used as tolerance index:

a) the acute toxicity of toxicant: Determination of LC_{50} 48 h (concentration of the toxicant which kills 50% of the test animals after 48 hours of exposure) according to the Bliss (1938) method (exp. 1 to 6a) and

b) a sublethal stress index: the respiration rate of animals (exp. 7 to 9a).

Respiration rates were determined potentiometrically with a Radiometer PHM 73 Blood Gas Analyser, using 20 ml disposable syringes as respiration chambers. The analyser chamber was connected to a Haake constant temperature circulator maintained at 22 °C throughout. Artemia were placed in syringes filled with test water. The syringes were carefully capped to exclude all air bubbles, incubated upside down in a darkened constant tempertature room and tested 3-5 hours later. Syringes were removed from the constant temperature room, inverted to disrupt possible oxygen gradients, and aliquots were injected directly into the Radiometer analyser chamber. Controls without Artemia exhibited negligible changes in oxygen concentration. The Radiometer electrode was calibrated prior to the first measurement. Readings in mm Hg were converted to percent saturation using the oxygen solubility tables of Truesdale and Downing (1954). Respiration rates in µl O_2 were calculated

Table I

The plan of the experiments

No. of experiment	Pollutant	Experiment animals (group)	Food	Pre-exposure concentration (fraction of LC_{50} 48 h)*	Duration of experiment (days)		Effects tested
					pre-exposure	detoxification	
1	oil	b	yes	1/300 1.	7	-	LC_{50} 48 h
1a	oil	b	yes	1/300 1.	7	7	"
2	Finasol OSR2	b	yes	1/1 1.	7	-	"
2a	Finasol OSR2	b	yes	1/1 1.	7	7	"
3	Oil/Finasol	b	yes	1/10 1.	7	-	"
3a	Oil/Finasol	b	yes	1/10 1.	7	7	"
4	Finasol OSR5	a	no	1/200 2.	2	-	"
4a	Finasol OSR5	a	no	1/200 2.	2	2	"
5	Finasol OSR5	a	no	1/200 2.	4	-	"
5a	Finasol OSR5	a	no	1/200 2.	4	4	"
6	Finasol OSR5	a	no	1/200 2.	6	-	"
6a	Finasol OSR5	a	no	1/200 2.	6	6	respiratory ratio rate
7	Finasol OSR5	a	no	1/200 2.	2	-	"
7a	Finasol OSR5	a	no	1/200 2.	2	2	"
8	Finasol OSR5	a	no	1/200 2.	4	-	"
8a	Finasol OSR5	a	no	1/200 2.	4	4	"
9	Finasol OSR5	a	no	1/200 2.	6	-	"
9a	Finasol OSR5	a	no	1/200 2.	6	6	"

* The LC_{50} 48 h of each toxicant is taken from Verriopoulos and Moraitou-Apostolopoulou (1983) and Christou (pers.com)

directly from the change in percent saturation before and after the experimental period. The respiration rate of acclimated and acclimated/detoxified Artemia was measured after exposure to 0.2 ppm of Finasol OSR5. Differences between the measured values of LC_{50} and respiration rates have been tested statistically by the t-test (significant = 95% level).

3. RESULTS

3.1 Acute toxicity experiments

Table II gives the LC_{50} 48 h values for the various experimental groups of Artemia and Fig. 1 the mortality curves of the experiments in which statistically significant changes in the LC_{50} 48 h values have been observed between the non-acclimated (controls) animals and the acclimated and acclimated/detoxified ones.

Table II

LC_{50} 48 h (in ppm) of the three toxicants to Artemia salina without and after pre-exposure to the same toxicants

Pollutant	Controls (non pre-exposed)	pre-exposed	pre-exposed/ detoxified
Oil	297.898	399.256 (exp. 1)	562.66 (exp. 1a)
Finasol OSR2	0.939	17.580 (exp. 2)	2.413 (exp. 2a)
Oil/Finasol OSR2	5.370	7.190 (exp. 3)	8.939 (exp. 3a)
Finasol OSR5	41.60	43.42 (exp. 4)	47.5 (exp. 4a)
"	"	41.85 (exp. 5)	42.02 (exp. 5a)
"	"	42.3 (exp. 6)	50.0 (exp. 6a)

3.1.1 Oil

An increase of the LC_{50}, compared with that of the control animals was noticed in the pre-exposed Artemia (exp. 1). The increase became more pronounced and statistically significant for the pre-exposed/detoxified animals (exp. 1a). The mortality curves showed that the pre-exposed Artemia were more resistant (lower mortality) to oil than the controls between 300 and 500 ppm of oil but not in lower oil concentrations and that the increase of resistance was more important at higher, and was lowered at lower concentrations. The detoxified Artemia were in all tested concentrations more resistant than the non pre-exposed animals. Comparing the resistance of detoxified and pre-exposed animals, the detoxified were less resistant to concentrations up to 450 ppm and more resistant over 450 ppm.

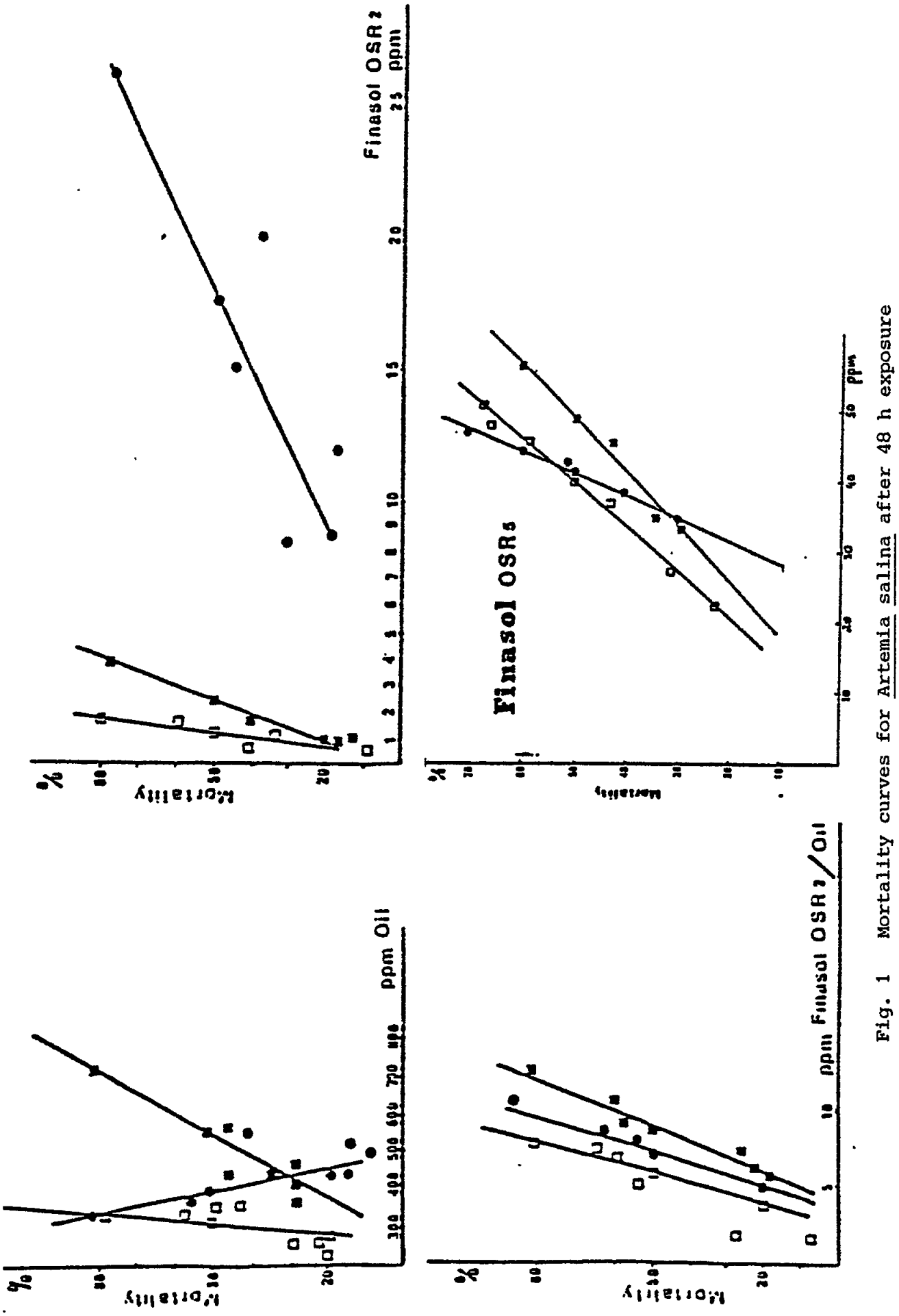

Fig. 1 Mortality curves for Artemia salina after 48 h exposure
(□ non-exposed, ● pre-exposed, ■ pre-exposed and detoxified)

3.1.2 Finasol OSR2

A very important and statistically significant increase of the LC_{50} was observed in the pre-exposed animals (exp. 2). The high resistance was weakened after the detoxification period but still the measured LC_{50} remained significantly higher than that of the non pre-exposed Artemia.

3.1.3 Oil/Finasol OSR2 mixture

An increase of the LC_{50} was observed both in the pre-exposed and in the pre-exposed/detoxified animals. This increase became more important in the pre-exposed/detoxified Artemia.

3.1.4 Finasol OSR5

Although an increase of the LC_{50} was noticed in all experimental groups (exp. 4 to 6a) only in the case of 6a experiment the increase was statistically significant. In all experiments the LC_{50} of the acclimated/detoxified Artemia was higher than that of the pre-exposed animals.

3.2 Sublethal effects (respiration rate)

The respiratory rates of non pre-exposed, pre-exposed and pre-exposed/detoxified Artemia after exposure to 0.2 ppm of Finasol OSR5 as well as the rate of non exposed (control) animals are shown in Fig. 2.

An abrupt, and statistically significant increase of respiration rate of Artemia was noticed after exposure of non acclimated Artemia to Finasol (group E1). This increase became less pronounced when pre-exposed animals were exposed to Finasol and an adaptation to Finasol proportional to the pre-exposure period was noticed. In the E2 group (exp. 7) it remains significantly higher than that of the controls. For the E3 and E4 groups (exp. 7,8) although the increase became even less important it still remains significantly higher than that of the controls. Finally in the E6 group (exp. 9) the increase is not significant.

The pre-exposed/detoxified groups (D2=exp. 7a, D4=exp. 8a, D6-exp. 9a) present also a proportional to the time of experiment (pre-exposure detoxification) lowering of their respiration rates compared with the rate of the non pre-exposed Artemia (group E1). The respiratory rates of acclimated/detoxified Artemia are significantly higher than that of the controls for the D2 group and non different for the D4 and D6 group.

4. DISCUSSION

From the results obtained it can be concluded that higher tolerance (acclimation phenomena, adaptation) to oil and oil dispersant, can be induced to Artemia salina after pre-exposure to these toxicants. The higher tolerance concerns both the acute toxicity effects (LC_{50}) and sublethal toxicity physiological disfunctions (respiration).

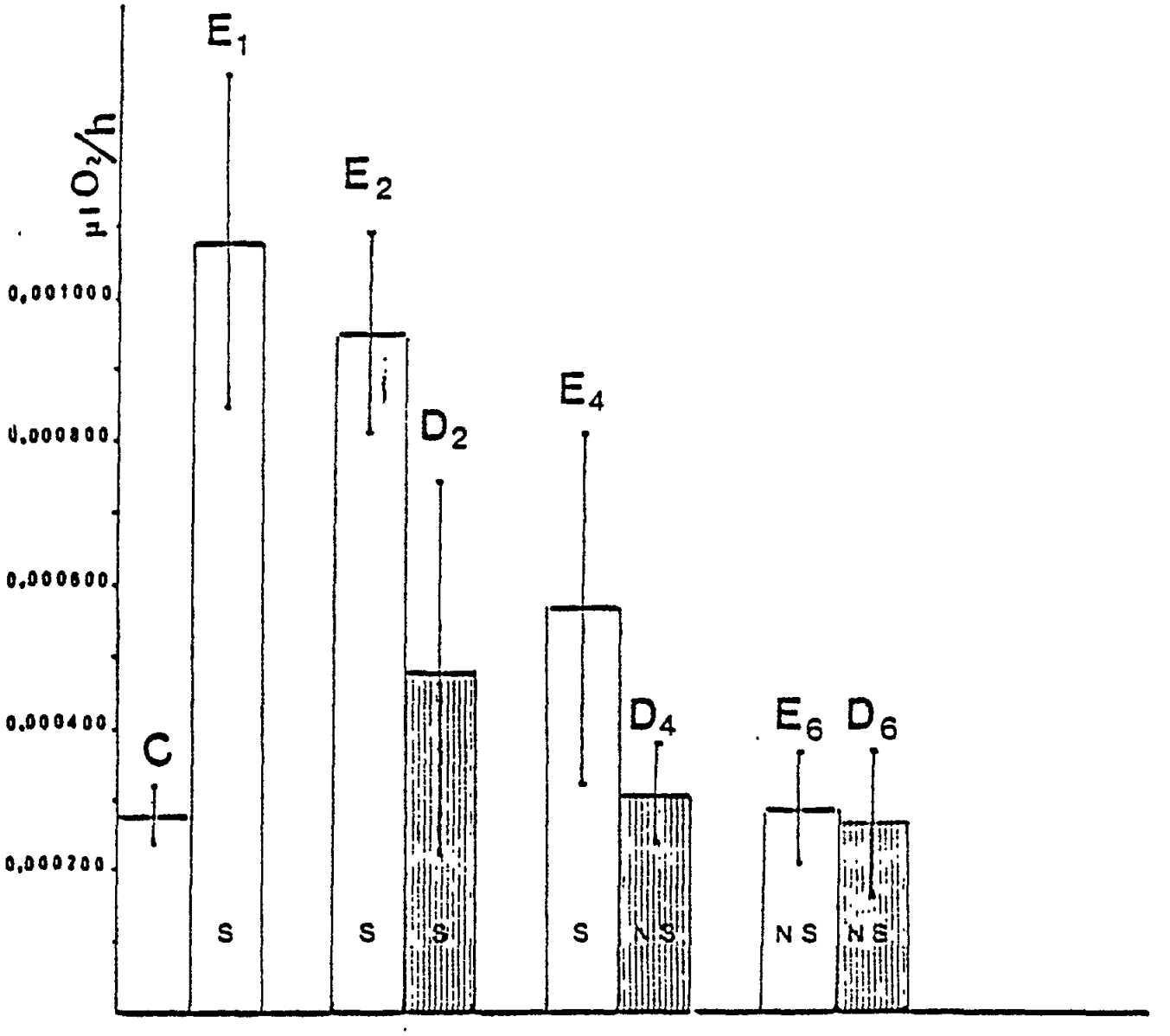

Fig.2 Respiration rates (µl O_2 h^{-1}) of the various experimental groups of Artemia salina after exposure to 0.2 ppm Finasol OSR5.

C = controls (non exposed animals)
E = non pre-exposed animals
E2 = pre-exposed (48 h)
E4 = " " (96 h)
E6 = " " (144 h)
D2 = " " (48 h) detoxified (48 h)
D4 = " " (96 h) " (96 h)
D6 = " " (144 h) " (144 h)
S = statistically significant change (95% level) compared with the rate of the controls (C)
NS = non significant rate of the controls (C)

Comparing the results of the various experiments it seems that factors of primordial importance for the establishment of acclimation to a toxicant are the concentration of the pre-exposure solution and the time of exposure. High pre-exposure concentrations lead to a rapid induction of acclimation phenomena (exp. 2) but the obtained higher resistance seems to be in part lost after exposure of the acclimated animals to clean sea water (exp. 2a). The higher tolerance of the Artemia pre-exposed to Finasol cannot be attributed to a genetic variability of Artemia because the same animals after the detoxification period presented a lowering of their tolerance to Finasol (Exp. 2a).

Exposure to low concentrations of the tested toxicants induce a slow appearance of adaptation phenomena. The obtained higher tolerance does not disappear after exposure to clean sea water but on the contrary seems to be strengthened after the detoxification period (exp. 1, 1a, 3, 3a). It could be concluded that in this case, adaptation proceeds through a slow but persistent mechanism. Similarly Moraitou-Apostolopoulou et at. (1983) have noticed that in Tisbe holothuriae acclimated to copper the effect of the pre-exposure to the respiration is not lost after exposure to clean sea water.

It seems that the toxicant concentration in the body of experimental animals is not a critical factor for the acquisition and persistence of acclimation. Generally it has been found that the uptake from solution in sea water is rapid, but after the animals are transferred to uncontaminated sea water large fractions of the hydrocarbons are quickly lost (Lee et al., 1972; Anderson et al., 1974). However small fractions remain which decrease very slowly. Furthermore, although it has been found that starvation is among the factors which change the rate of absorption of a contaminant, no differences could be detected in the process of adaptation phenomena between fed and unfed animals.

Concerning the minimum time needed for the induction of adaptation phenomena, it seems that the slow proceeding adaptation mechanism needs (for Artemia and for the tested concentrations) about 12 days with no obligatory exposure to the toxicant during all that period, while in our high pre-exposure experiments, acclimation was already produced at the end of the experimental period (7 days).

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ACUTE TOXICITY TESTS WITH DISPERSANTS AND OIL DISPERSANT MIXTURES

by

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A B S T R A C T

This project was carried out for short term toxicity tests using dispersants and oil/dispersant mixtures (1:10) on two populations of Artemia; a parthenogenetic one and an amphigonic (nauplii instar I and II-III). LC_{50} determinations were carried out for a minimum of 6h and a maximum of 48h.

The common method which is used in order to determine LC_{50} was proved to be non-effective for some of the polluting substances. Therefore we proceeded to a qualitative spectrophotometric project in order to clarify the reasons which cause the inability in determining LC_{50} for those dispersants.

The results of the spectrophotometric analysis gave us satisfactory explanations to the problems which appeared during the toxicity tests.

The harmful effect of the polluting compounds under constant laboratory conditions using an electron microscope was verified.

1. *INTRODUCTION*

The problem of marine pollution is of great importance, especially for the Mediterranean countries due to their extensive coastal areas as well as the slow renewability of the sea water.

Oil spills are a serious threat for the fauna and flora of the sublittoral zone, having a direct effect on the economics of fisheries and tourism. This problem was designated as a local one until the 1st World War. Since then, technological advances and industrial development have been closely associated with the accidental release of oil. The number of accidents through oil spills and leakages during transportation have increased a great deal over the last few years and, in many cases, have irreparably affected the existing balance among the ecosystems.

Hydrocarbon toxicity is not limited to short-term phenomena like immediate death, but also long-term effects.

It is possible for the poly-nucleic aromatic hydrocarbons to remain in the tissues of the organisms which have been exposed to petroleum, to achieve such high concentrations along the "food chain" as to threaten man as a consumer. A number of methods have been employed to check and control this kind of pollution. One of them is the use of surfactants which disperse oil after contact with it.

The first generation of these dispersing compounds was highly toxic, and thus the short-term toxicity tests were of great importance. The newer generations of the surfactants are considerably less toxic or almost non toxic (completely harmless). Furthermore, other experimental methods have had to be employed on the normal level of organisms and in doses well below the "lethal" one (sublethal).

There are many projects dealing with acute toxicity tests. This project was carried out for acute toxicity tests using dispersants and their mixtures with hydrocarbons (ratio 1:10).

It must be noted that the ratio used was kept constant for all the concentrations tested. Spectrophotometry was used for the analysis of some test solutions where the exact determination of the LC_{50} was impossible. At the same time we verified the harmful effect of the polluting compounds under constant laboratory conditions by using an electron microscope (sublethal toxic effects).

2. MATERIALS AND METHODS

(a) Artemia as a test animal

The Artemia species were selected for short term toxicity tests taking into account that:

The cysts may survive in the laboratory for long-time intervals: 24 hours after hatching, the young animals are obtained which then can be easily cultured (Sorgeloos, 1974; Vanhaecke et al., 1980). Their biological cycle is well known as well as their breeding (Sorgeloos, 1980). They are quite sensitive to the chemical variations of the experimental medium and this sensitivity depends on their geographical origin (Claus et al., 1977; Castritsi-Catharios et al., 1984; Castritsi-Catharios (in press)) and the age of the animals (Vanhaecke et al., 1980).

A great number of animals need only a small quantity of the medium in order to survive. From the economical point of view, they seem to be very attractive and of great interest as food for fish larvae and crustaceans. Finally their biotopes are also located in the Mediterranean whose mild climate is very suitable for their development.

Due to the above reasons, Artemia was used as a test animal in our experiments. A parthenogenetic strain coming from the Greek saltworks of Missolonghi were used (Fig.1 illustrates a map of the area where the sampling has taken place), As well as amphigonic strains supplied by the Carolina Biological Supply Company, USA.

The processing of the cysts was carried out according to the method described by Sorgeloos (1980). For the short-term experiments only larvae (nauplii) instar I and II-III were used (Claus, 1976; Sorgeloos, 1978; Sorgeloos et al., 1978;) which are more sensitive. For long-term tests Artemia adults are the most suitable experiment animals (Tarpley, 1958; Brown and Absanullah, 1971; Saliba and Krzyz, 1976).

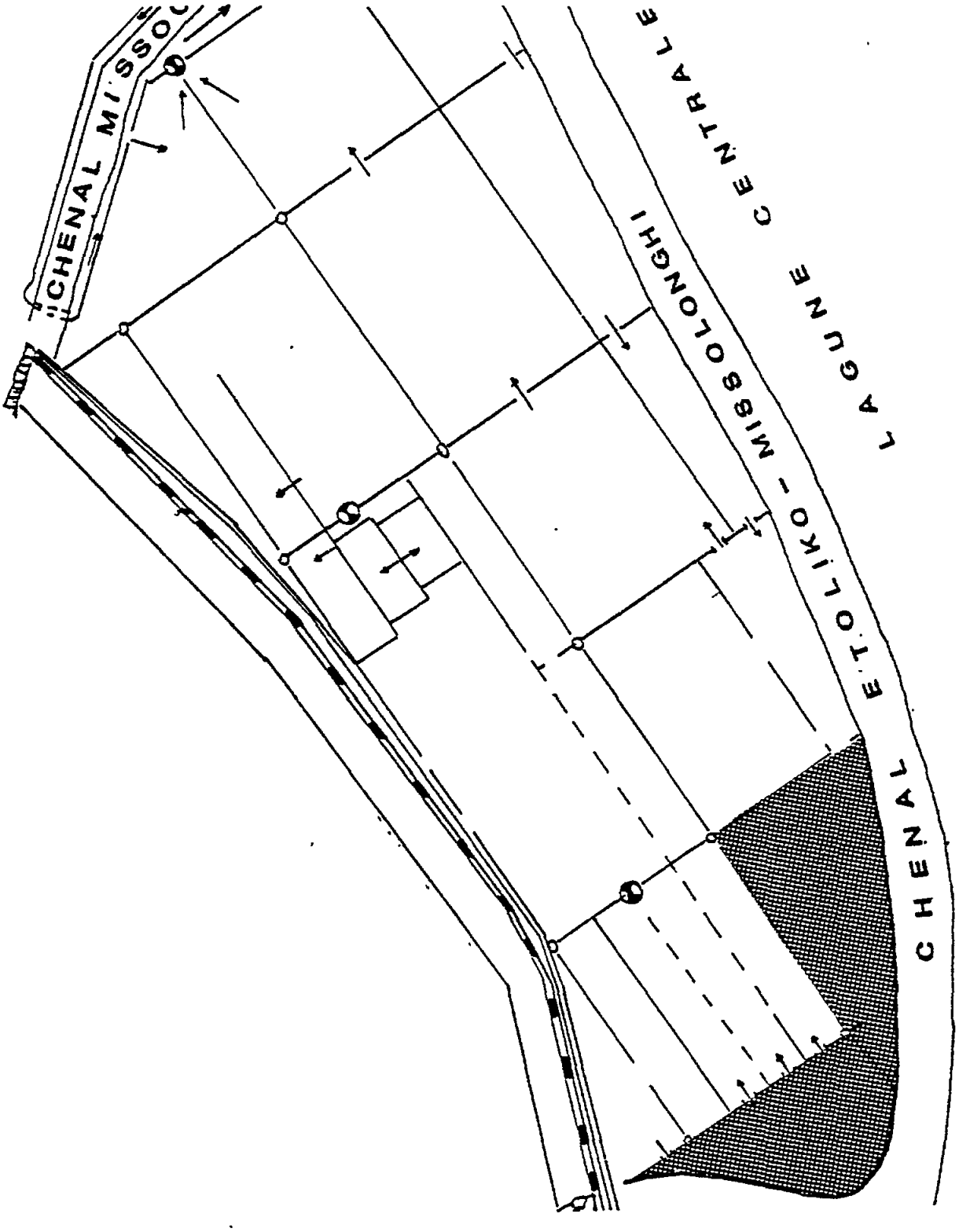

Fig. 1 Saltworks of Missolonghi where the Artemia sampling has taken place

At the very beginning of each experiment, it is essential that all the animals are of the same age (Tarzwell, 1969). That explains why the hatching of the cysts took place under absolutely controlled conditions (temparature, illumination and pH) and then fast selection of the nauplii was done by a binocular stereoscope and observing their brown - orange colour caused by the yolk presence.

(b) Test duration

The duration of the experiments carried out did not exceed the time interval of 48 hours because, beyond that point, the natural mortality of the test animals increases considerably (eg: 36% natural mortality for 72 hours). During this time the Nauplii were being fed from the yolk, therefore, during the experiments, the feeding of the animals should be avoided because it could lead to inaccurate results.

Finally the required pollutant concentration is achieved within this short time interval, avoiding the "continuous flow" system.

(c) Polluting substances and media

Clean sea water was used after double filtration. Salinity and pH were measured to verify the corresponding values of $35^{o}/oo$ and 8.1 respectively. An initial pollutant solution was prepared, through which, by proper dilution, the required concentrations were obtained. The correction of the pH of the medium was almost continuously done during the process of hatching. As it is known, alkaline water positively affects the percentage of hatching. The calibration was achieved with the addition of 2.4×10^{-4}M solution of $NaHCO_3$.

The pH of the natural sea water used for the spectrophotometric analysis, was at the same (more or less) level with the one used for the toxicity experiments, (The difference between them was about $\pm$ 0.15 pH units). No calibration of the two pH values was done since the difference between these two values was negligible and the ionic nature of the chemicals which would be used for the correction might affect the solubility of the dispersants.

The pH readings for the water used in the toxicity experiments was determined using a Broadley-James pH-meter, model 10512, equipped with a BJC polymerism electrode.

The pH readings for the water used in the spectrophotometric experiments came from a "CORNIG model 7" pH-meter equipped with a BJG polymerism electrode. Each reading was taken 5 min. after the electrode had been immersed into the sample (Table I).

From the data in Table I, it can be easily seen that the more concentrated solutions appear to be less alkali in comparison with the more diluted. This proves that surfactants are ionised and behave like weak bases.

Dispersants are in principle mixtures of multiethoxylised compounds in isopropanol solutions, aromatic hydrocarbon solutions and other additives. In the newest generation of dispersants the hydrocarbons content is reduced since the toxicity was actually due to the hydrocarbons. Theoretically, they are biodegradable and of very low toxicity for the marine organisms.

Their molecules consist of a lyophilic and a lyophobic part, so they have got the ability to scatter in oil and water respectively. They are able to disperse oil because of the adsorptial capacity of the micelles, which is also characteristic of the colloid solutions. The foam which is produced during the quick mixing of high concentration dispersant solution, is a colloid system of a gas dispersed within a liquid. In these systems the gas bubbles are distributed within a liquid separated by a thin membrane whose dimensions are usually equal to those of the colloid micelles.

Table I

pH changes of the sea water after dispersant addition.

Dispers./ water ratio	OSR2		OSR4		OSR5		OSR7		
	1/1000	1/5000	1/1000	1/5000	1/1000	1/5000	1/1000	1/5000	H_2O
Temperature	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5
pH(dispers/ H_2O	8.50	8.55	8.40	8.50	8.65	8.70	8.60	8.70	8.15
pH increase after addition of dispersant	0.35	0.40	0.25	0.35	0.50	0.55	0.45	0.55	-

(d) Preparation of experimental solutions

The dispersants used for the preparation of the experimental solutions were measured volumetrically, in order to eliminate any error caused in the concentration reading due to loss of the toxic compound while being transferred from the weighing container to the washing container and then to the volumetric vessel. Therefore precision pippetes were used for the introduction of the dispersant to the solution.

The volume of the dispersant required for the preparation of the initial solution was calculated through the following formula:

$$V = \frac{B}{(e.b.)}$$

where: V= volume of dispersant.

B= dispersant weight.

e.b.= specific weight 0.958 gr/cm^3.

The stirring time of the initial solution was 15 min. For each new concentration (prepared from the initial solution by dilution) stirring time was 30 min. at the same speed (1 000 rpm).

(e) Specific weight of dispersants

The determination of the specific weight of each dispersant was obtained by weighing ten samples of equal volume (1 ml), and then working out the average weight. (Table II).

Table II

Determination of dispersant specific weights.

Dispersant	OSR2	OSR4	OSR5	OSR7
Temperature	22.5°C	22.5°C	24°C	23.5°C
S.W. gr/cm^3	0.779	0.787	1.007	0.958

The variation of the specific weight with respect to temparature is shown in Table III.

Table III

Variation of specific weight of dispersants with temperature.

T °C	OSR2	OSR4	OSR5	OSR7
11.00	-	-	1.031	0.990
13.50	0.794	0.809	-	-
16.50	-	-	1.019	0.985
18.00	0.788	0.804	1.013	0.975
19.50	0.785	0.802	-	-
20.00	-	0.801	1.009	0.971
22.50	0.779	0.787	-	-
23.50	0.777	-	-	0.958
24.00	-	-	1.007	-

(f) Acute Toxicity Tests

During the accute toxicity tests we have used:

(a) dispersants Finasol OSR2, OSR5, OSR7, BP 1100X in their concentrated form. (b) mixtures of the above dispersants with gas-oil, crude-oil and mobil-oil at a constant ratio of 1:10.

For each concentration we have used 100 animals (10 per petri dish). These 10 nauplii were transferred by using a pasteur pipette marked so that 0.1 ml of liquid could be handled. By knowing the exact quantity of the water transferred to the toxic mixture we could exactly calculate the alteration of the solution concentration. This way the possibility of error was eliminated ($N = \frac{10\ No}{10.1}$).

During each experiment, lighting, temparature and number of animals were kept constant.

The statistical analysis of the results obtained from the tests was performed by using the "Bliss" method which was incorporated in a computer program especially designed to meet the requirements of this particular research study. The program was written in "Ansi-Fortran 4".

Since in some experiments the results were not reproduced with acceptable tolerance, and the LD_{50} was not calculated with good accuracy, we went on by studying the solutions spectrophotometrically.

(g) Spectrophotometry

For the spectrophotometry tests, the following two types of spectrophotometers were used:

Beckman DB - G.
Varian DMS - 80

The difference between the readings taken through the above two types of instruments ranges within ($\pm$ 1) plus or minus one.

Quartz cells were used allowing UV radiation to pass through. After the spectra of the OSR2, OSR4, OSR5, and OSR7 dispersant solutions were obtained, the greater absorption was measured. A close scanning every 1 nm was then carried out in order to determine the radiation wave length wherever a "peak" existed.

The values of Table IV were used as standard values for spectrophotometry tests of the dispersant solutions.

The indications on the greater wave-lengths of the ultra-violet part (300-400nm) were very stable and were repeated for a large number of times. Since the "Lambert - Beer" law is valid only for cases of thin solutions, the experiments were carried out using solution concentrations less than 200 ppm. For the preparation of the solutions the same procedure was followed as the one employed in the toxicity tests. The stirring time of the initial solution was for 11 min. whereas the stirring time of solutions prepared from it, was 30 sec. at the constant speed of 1000 rpm.

(h) Experiments carried out using the electron microscope

The effects of the toxic solutions on the digestive epithelium of the Artemia nauplii at stage II-III (strains of Missolonghi and Carolina) was observed in relation to the Finasol OSR5 dispersant and the OSR5/Gas oil mixture.

Table IV

Transmittance values of the dispersants OSR2, OSR4, OSR5 and OSR7 measured in several wavelengths (353-368 nm).

nm	OSR2 T	OSR4 T	OSR5 T	OSR7 T
353	61.85	69.60	95.10	64.45
354	61.85	69.65	95.15	64.50
355	62.90	69.70	95.20	64.60
356	62.95	69.75	95.30	64.65
357	63.00	69.80	95.40	64.70
358	62.95	69.85	95.50	64.70
359	63.00	69.90	95.60	64.75
360	63.25	69.95	95.65	64.75
361	63.40	69.95	95.70	64.75
362	63.35	70.00	95.75	64.80
363	63.40	70.05	95.80	64.80
364	63.00	70.05	95.85	64.85
365	63.05	70.10	95.90	64.85
366	63.10	70.15	96.00	64.90
367	63.20	70.20	96.05	64.90
368	63.90	70.25	96.10	64.95

* The linear correlation appeared on the visual part of the spectrum obliged us to use the U-V part of it in our measurements.

The animals had been taken out from lethal and sublethal concentrations as well from natural sea water. The materials used for their consolidation and storage were the following:

A Resin: Epon - 812, Aralolite 502, D.O.S.A.

B Resin: A Resin + DMP - 30.

Regulating solution: sodium cacodylic 0.08M (pH= 7.4).

A; consolidative: 2% paraphormaldehyde, 2% gluterinaldehyde in 0.08M sodium cacodylic (pH = 7.4).

Washing liquid: Sucrose in 0.1M sodium cacodylic in 0.08M (PH = 7.4).

B; consolidative: 2% Osmium tetroxide (OsO4).

Sections of 300-600 $_A$ thickness were produced on a supermicrotome, and then placed on fine 200 mesh copper net, 3 diameter. The dyeing of the sections was accomplished by using 7% acetic uranyle (U.A.) and citric lead.

3. RESULTS AND DISCUSSION

A number of different tests were performed for a wide source of concentrations prior to the final experiments. Results are reported below.

The results obtained for both the parthenogenetic and the amphigonic strains of LC_{50} are given in Table V. An example is also provided showing the method used for the LC_{50} in the Finasol/Gas oil mixture at temperatures between 25 ^{o} and 27 ^{o}C. The test animal used was Artemia from Missolonghi.

Table V

Lethal concentration (ppm) of the Finasol OSR5, OSR2, Finasol OSR5/Gas oil for the strains tested.

FINASOL OSR5

Artemia from Missolonghi st. I		Artemia Francescana, st. II	
LC^{6}_{50}	LC^{24}_{50}	LC^{6}_{50}	LC^{24}_{50}
7.453 ppm	258.8 ppm	335 ppm	44.8 ppm

FINASOL OSR5/Gas oil 1:10

LC^{6}_{50}	LC^{24}_{50}	LC^{6}_{50}	LC^{24}_{50}
2.675 ppm	653.8 ppm	1.203,1 ppm	338 ppm

FINASOL OSR2

LC^{48}_{50}	LC^{24}_{50}
217 ppm	222.4 ppm

Furthermore it must be pointed out that it was not possible to determine the precise amounts of LC_{50} in the (1) BP1100X/crude oil, (2) Finasol OSR7/Mobil oil and (3) OSR7 mixtures.

Further, below are given the concentrations in ppm and the mortality percentages of the Artemia from Missolonghi at stage II - III exposed under the above polluting compounds.

1st experiment

Concentration in ppm :	0	100	200	300	400	500	600	700	800	900	1000
% mortality after 6 h:	0	0	0	0	0	0	0	0	0	0	0
% mortality after 24h:	0	0	0	0	0	0	0	0	0	2	2

2nd experiment

Concentration in ppm :	0	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
% mortality after 6 h:	0	0	0	0	0	0	0	2	2	4	4
% mortality after 24h:	0	2	2	2	2	2	2	2	4	4	4

3rd experiment

Concentration in ppm :	0	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
% mortality after 6 h:	0	0	0	0	0	0	2	2	4	4	6
% mortality after 24h:	0	0	0	0	0	0	2	4	4	6	6

Mixtures having concentrations above 10,000 ppm are considered non-toxic and the solutions of very high concentrations were not uniform, so further experiments were not carried out.

The main purpose of employing spectrophotometry was not the examination of the qualitative behaviour of dispersants in water solutions, but the approximate determination of the reasons causing some dispersants to behave abnormally in our toxicity tests.

The dispersants which were studied spectrophotometrically are the ones appearing in the following tables (Table VI) and in Figs. 2, 3, 4, 5 and 6.

During the mixing of the dispersants with the sea water the following were observed:

(a) Reduction in the volume of the solution due to the absorption phenomena and change in the thermal equilibrium;

(b) Foam generation during mixing;

(c) Appearance of a superficial film by the time.

This implies a reduction in concentration of the solution due to the reduction appearing in absorption by the time. The timing and the way of mixing the solutions, determines the number and the size of the colloid particles generated. It also determines the size of the active surface and hence toxicity.

As far as OSR2 and OSR4 dispersants are concerned a reduction in absorption was observed with the passage of time. This effect was caused by a quantity of the dispersant that came to the surface due to the destruction of the existing colloidal situation. In the OSR5 dispersant a reduction was observed in the absorption of the solution after an interval of 6 hours.

However after 24 and 48 hours a new increase in absorption was observed. Also in the OSR7 the absorption increased after an interval of 24 hours and decreased again after about 48 hours. The instability which governs all colloid systems was also very obvious in the OSR7 solutions.

The existing dynamic equilibrium situations are metastable and the effects observed are most probably due to:

- Chemical reactions taking place within the solution
- Change in the mycell size which varies the percentage of light diffusion
- Variation of the absorption degree due to changes in pH and temperature, or effect of light.

Table VI

Correlation between absorbance (nm), transmission (%) and concentration (ppm) for various degradation times. Dispersants: OSR2, OSR4, OSR5, OSR7.

OSR2

hrs	0		6		24		48		
No	A	%T	A	%T	A	%T	A	%T	ppm
1	0.857	19.90	0.761	17.30	0.378	41.85	0.378	41.85	200
2	0.838	14.52	0.745	18.00	0.367	43.00	0.355	44.22	188.89
3	0.786	16.40	0.729	18.65	0.357	44.1	0.347	44.90	177.78
4	0.740	18.20	0.688	20.60	0.346	45.1	0.275	53.35	166.67
5	0.694	20.25	0.655	22.20	0.332	45.50	0.248	56.50	155.56
6	0.648	22.50	0.618	24.15	0.316	48.25	0.227	59.30	144.44
7	0.600	25.1	0.568	27.00	0.299	50.25	0.201	62.85	133.33
8	0.545	28.65	0.528	29.70	0.286	51.80	0.181	66.0	122.22
9	0.505	31.2	0.485	32.80	0.269	53.80	0.165	68.3	111.11
10	0.457	34.85	0.448	35.60	0.250	56.25	0.158	69.55	100.00
11	0.408	39.05	0.391	40.15	0.230	58.85	0.139	72.60	88.89
12	0.356	44.10	0.343	45.30	0.214	61.1	0.122	75.50	77.78
13	0.306	49.15	0.286	51.80	0.193	64.0	0.112	77.25	66.67
14	0.260	54.95	0.238	57.80	0.172	67.30	0.105	78.60	55.56
15	0.212	61.40	0.183	65.60	0.149	70.95	0.088	81.65	44.44
16	0.158	69.50	0.125	74.95	0.121	75.70	0.072	84.75	33.33
17	0.018	77.95	0.086	82.00	0.079	83.35	0.053	88.44	22.22
18	0.051	88.90	0.036	92.2	0.036	89.95	0.037	91.65	11.11

OSR4

hrs	0		6		24		48		
No	A	%T	A	%T	A	%T	A	%T	ppm
1	0.702	19.85	0.398	40.00	0.348	44.85	0.264	54.60	200
2	0.581	26.25	0.321	47.75	0.301	50.00	0.229	59.05	188.89
3	0.477	33.35	0.281	52.35	0.269	53.85	0.182	65.75	177.78
4	0.382	41.50	0.232	58.60	0.227	59.30	0.163	68.70	166.67
5	0.321	47.75	0.197	63.55	0.193	64.10	0.248	71.10	155.56
6	0.278	52.70	0.179	66.25	0.170	67.60	0.129	74.30	144.44
7	0.240	57.55	0.166	68.25	0.156	69.80	0.121	75.70	133.33
8	0.216	60.80	0.155	70.00	0.144	71.80	0.110	77.60	122.22
9	0.197	63.55	0.138	72.80	0.131	73.95	0.099	79.60	111.11
10	0.176	66.70	0.125	75.00	0.117	76.40	0.088	81.65	100.00
11	0.155	70.00	0.111	77.45	0.102	79.10	0.079	83.35	88.89
12	0.138	72.80	0.094	80.55	0.092	80.90	0.069	85.30	77.78
13	0.119	76.05	0.081	83.00	0.079	83.35	0.058	87.50	66.67
14	0.097	80.00	0.068	85.50	0.065	86.10	0.050	89.15	55.56
15	0.078	83.55	0.056	87.90	0.054	88.30	0.040	91.20	44.44
16	0.059	87.30	0.039	91.40	0.038	91.65	0.030	93.35	33.33
17	0.038	91.65	0.028	93.75	0.025	94.40	0.021	95.30	22.22
18	0.020	95.50	0.014	96.85	0.012	97.25	0.010	97.70	11.11

OSR5

hrs	0		6		24		48		
No	A	%T	A	%T	A	%T	A	%T	ppm
1	0.203	62.65	0.323	47.55	0.392	40.55	0.332	46.55	200
2	0.179	66.20	0.287	51.65	0.380	41.65	0.321	47.75	188.89
3	0.157	69.65	0.251	56.10	0.371	42.55	0.312	48.75	177.78
4	0.137	72.95	0.212	61.35	0.370	42.65	0.299	50.25	166.67
5	0.119	76.05	0.160	69.20	0.369	42.75	0.298	50.35	155.56
6	0.103	78.90	0.131	73.95	0.359	43.75	0.293	50.95	144.44
7	0.090	81.30	0.099	79.60	0.344	45.29	0.290	51.30	133.33
8	0.081	83.00	0.079	83.35	0.323	47.53	0.288	51.50	122.22
9	0.073	84.50	0.068	85.50	0.301	50.00	0.281	52.35	111.11
10	0.066	85.90	0.060	87.10	0.268	53.95	0.274	53.20	100.00
11	0.058	87.50	0.052	88.70	0.223	59.84	0.243	57.15	88.89
12	0.052	88.70	0.044	90.35	0.180	66.05	0.216	60.80	77.78
13	0.043	90.55	0.036	92.05	0.132	73.80	0.189	64.71	66.67
14	0.035	92.25	0.031	93.10	0.095	80.35	0.155	69.98	55.56
15	0.028	93.75	0.023	94.85	0.073	84.55	0.125	75.00	44.44
16	0.021	95.30	0.018	95.95	0.046	89.95	0.091	81.10	33.33
17	0.013	97.05	0.012	97.25	0.026	94.20	0.062	86.70	22.22
18	0.007	98.40	0.006	98.65	0.008	98.15	0.028	93.75	11.11

OSR7

hrs	0		6		24		48		
No	A	%T	A	%T	A	%T	A	%T	ppm
1	0.170	67.60	0.250	56.25	0.304	49.65	0.260	54.95	200
2	0.162	68.85	0.231	58.75	0.284	52.00	0.234	58.35	188.88
3	0.153	70.30	0.219	60.40	0.264	54.45	0.213	61.25	177.78
4	0.143	71.94	0.201	62.95	0.240	57.55	0.190	64.55	166.67
5	0.132	73.80	0.197	63.55	0.221	60.10	0.172	67.30	155.56
6	0.124	75.15	0.180	66.05	0.202	62.80	0.161	60.00	144.44
7	0.112	77.25	0.165	68.40	0.183	65.60	0.152	70.45	133.33
8	0.102	79.10	0.151	70.65	0.168	67.90	0.137	72.95	122.22
9	0.094	80.55	0.136	73.10	0.149	70.95	0.125	75.00	111.11
10	0.084	82.40	0.119	76.05	0.132	73.80	0.112	77.25	100.00
11	0.073	84.55	0.108	78.00	0.117	76.40	0.099	79.60	88.89
12	0.064	86.30	0.091	81.10	0.103	78.90	0.086	82.05	77.78
13	0.054	88.30	0.075	84.15	0.089	81.50	0.073	84.55	66.67
14	0.045	90.15	0.069	85.30	0.077	83.75	0.062	86.70	55.56
15	0.034	92.45	0.054	88.30	0.061	88.90	0.047	89.75	44.44
16	0.022	95.05	0.038	91.60	0.045	90.15	0.035	92.25	33.33
17	0.014	96.85	0.093	94.85	0.027	94.00	0.023	94.85	22.22
18	0.005	98.85	0.009	97.95	0.012	97.30	0.010	97.70	11.11

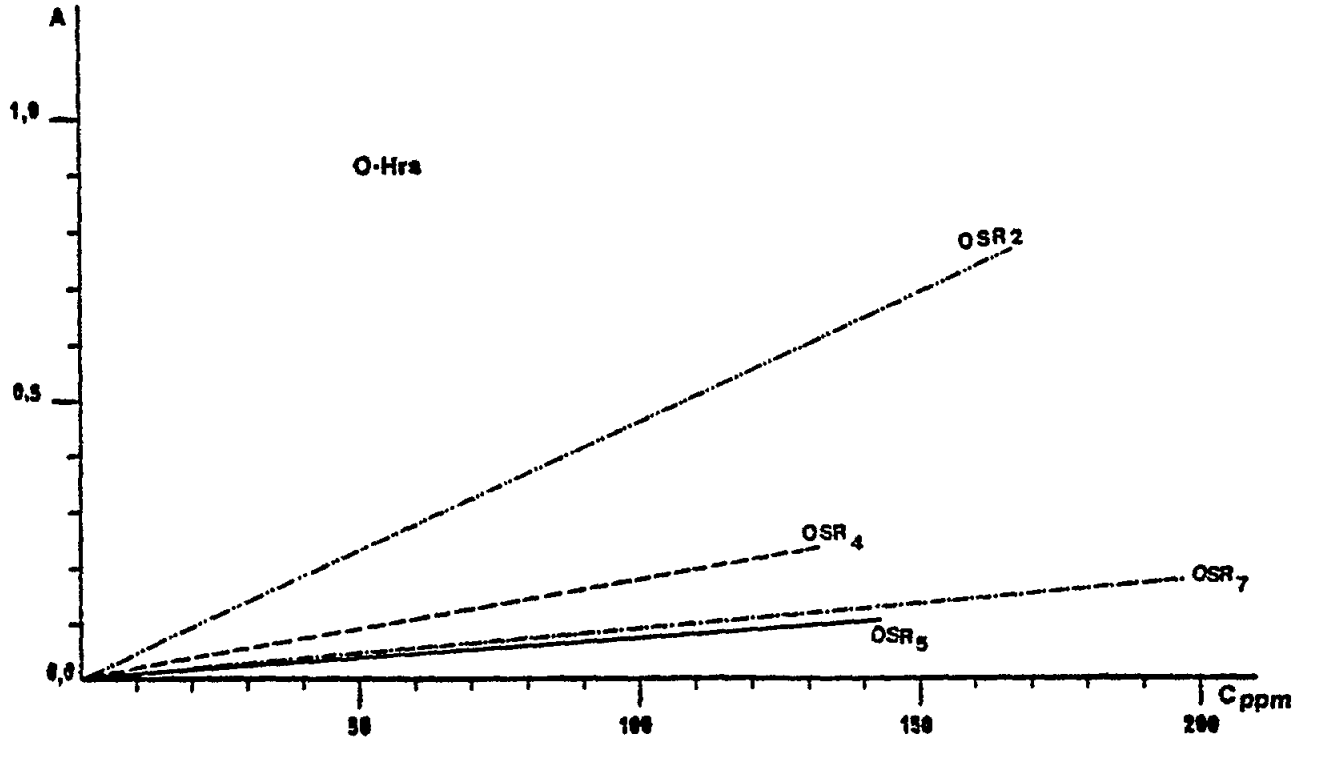

Fig. 2 Correlation between concentration (ppm) and absorbance (nm) for various dispersants. Degradation time t = 0h.

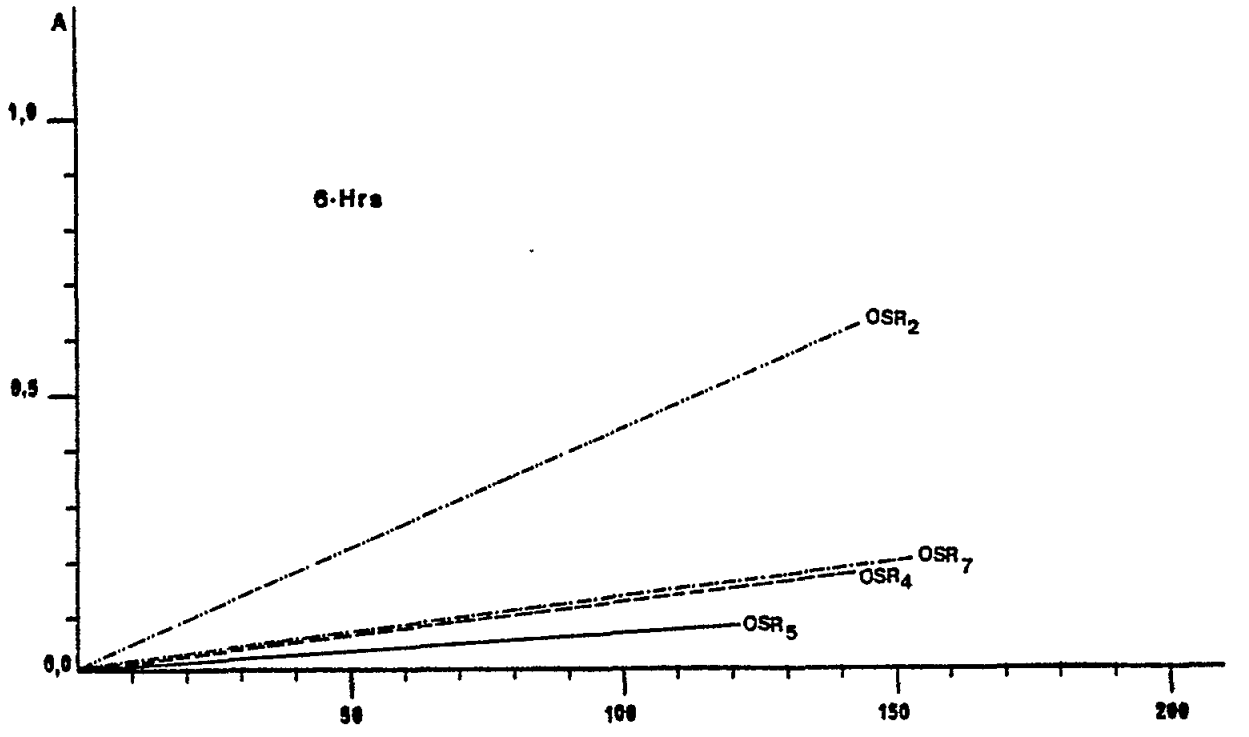

Fig. 3 Correlation between concentration (ppm) and absorbance (nm) for various dispersants. Degradation time t = 6h.

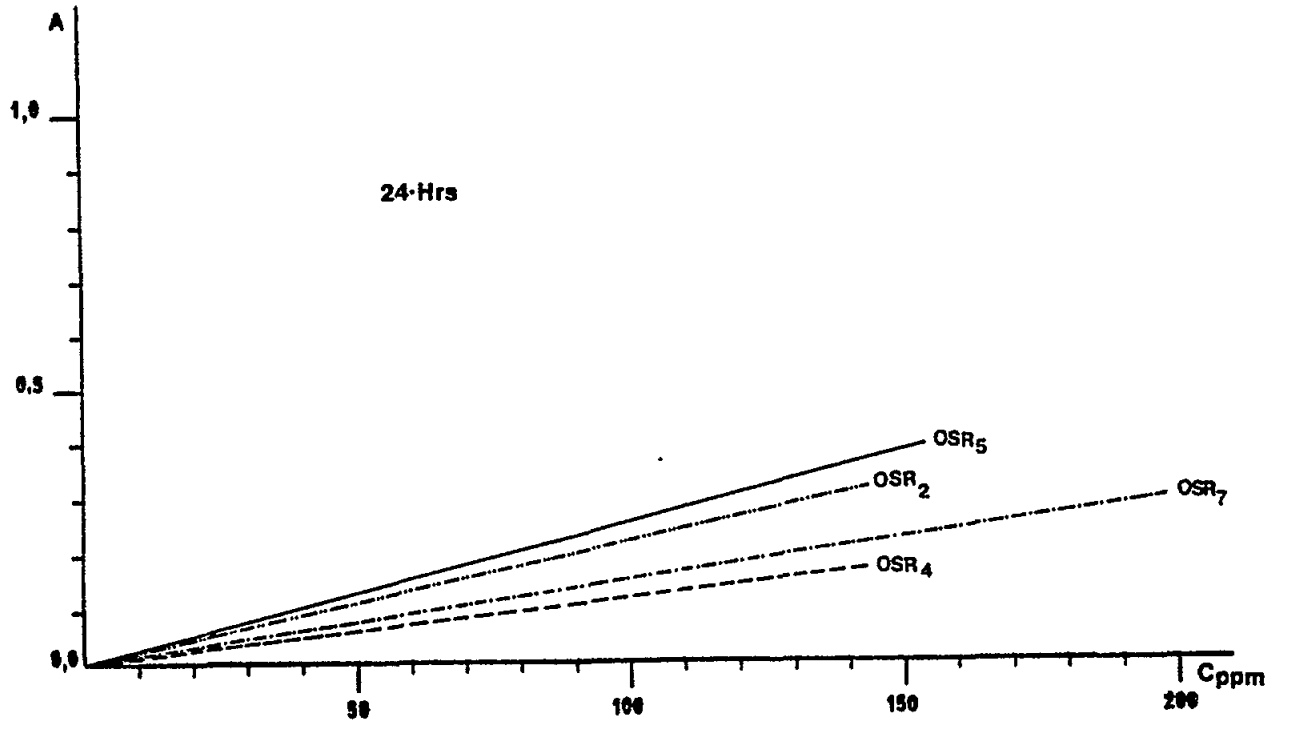

Fig. 4 Correlation between concentration (ppm) and absorbance (nm) for various dispersants. Degradation time t = 24h.

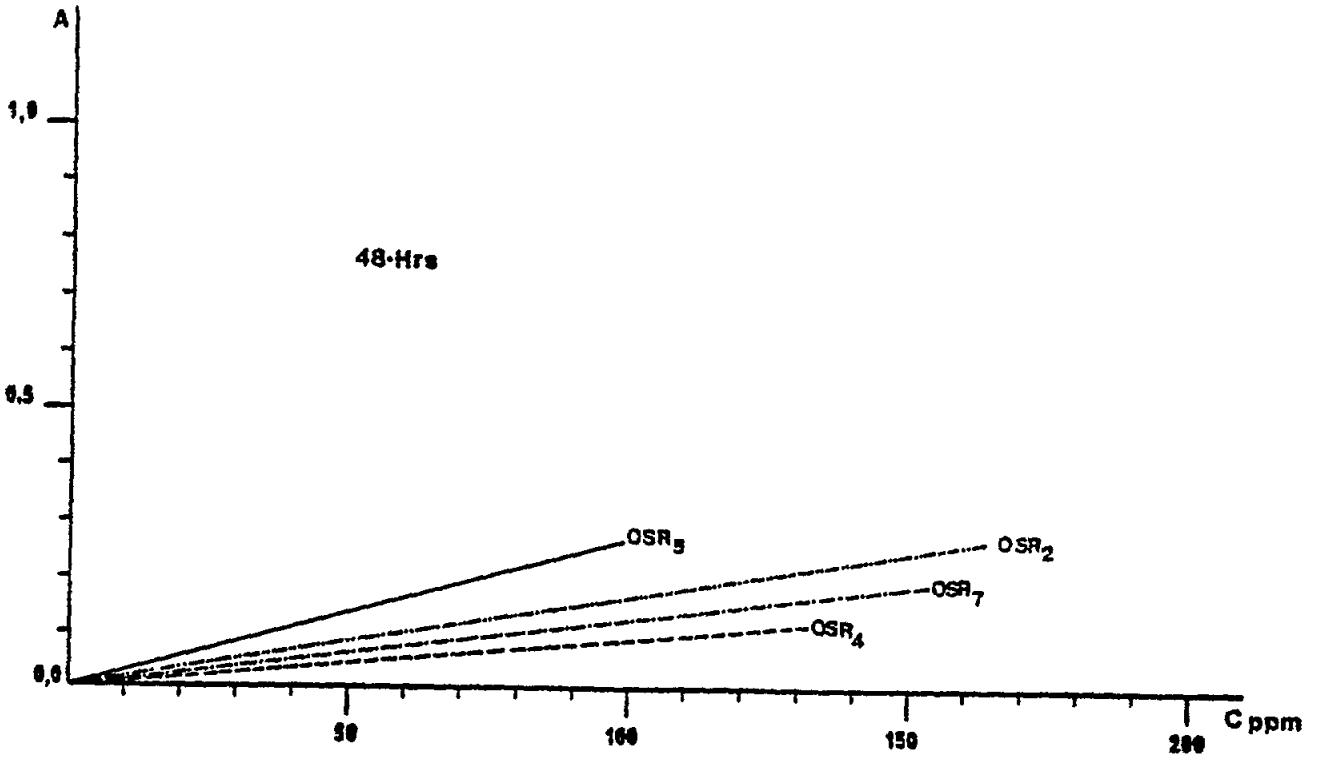

Fig. 5 Correlation between concentration (ppm) and absorbance (nm) for various dispersants. Degradation time t = 48h.

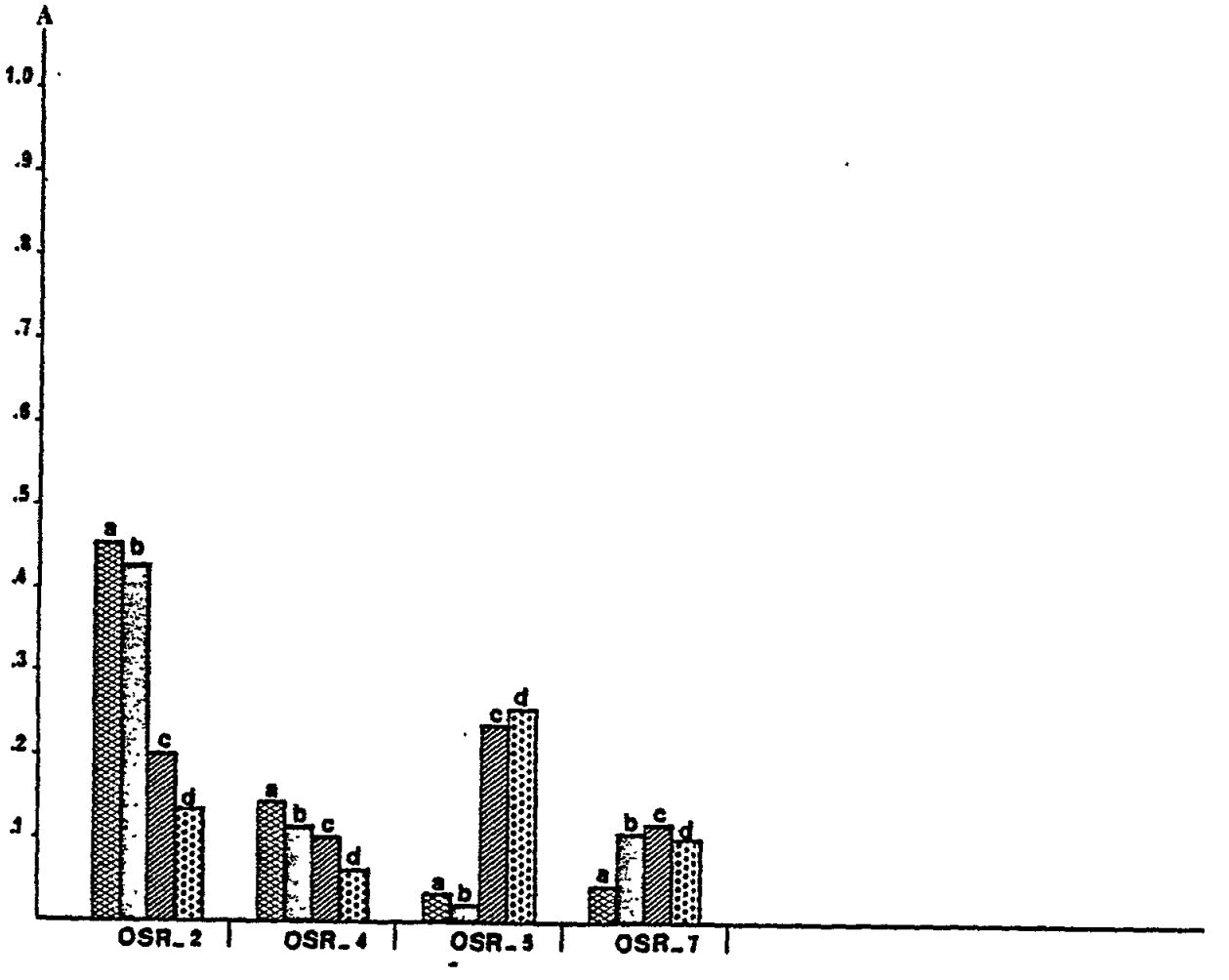

Fig. 6 Correlation between absorbance (nm) and degration time for various dispersants. Concentration = 100 ppm, a = 0hrs, b = 6 hrs, c = 24 hrs, d = 48 hrs.

All the above observations justify quite clearly our inability to determine precisely the LC_{50} (lethal concentration) of the OSR7. The fact that the abnormality was observed in the newer dispersants, leads to the conclusions that this probably occurred due to the peculiarity in the manufacture of the dispersants designed to render them self-destructive.

The experiments performed with the OSR2 dispersant, are mainly a repetition of the older ones (Castritsi-Catharios et al., 1980, 1982). That repetition was judged as necessary since materials had been stored in the state warehouses for more than five years.

As was observed, there was a difference between the two sets of results due to the following factors:

1. Oil dispersants are self-disrupted by manufacture;

2. The Artemia strain used was not the same in both cases. The differentiation of their sensitivities has already been pointed out at greater length;

3. The experimental conditions were not the same in both instances.

Fine structure of the alimentary mesenderon epithelial cells in Artemia salina stage II Nauplii

The mesendero of the alimentary tract in Artemia larva and adult is the region of water absorption. For this reason Artemia was selected for the experiments using electron microscopy. The mesendero epithelial structure is characteristic. An abundance of microvilli enables the topographic distinction. Their role is to increase the active surface of the cell without a simultaneous increase of its volume. The microvilli of the mesendero have approximately the same structure as those which are present in insects, even though they assumed the characteristic mucopolysacharride complex.

The structure of normal epithelial cells are observed in the control by means of electron microscopy and according to the description includes a large nucleus and one or two nucleoli. Around the nuclear membrane an abundance of ribosomes is observed. The yolk platelets which occupy the larger surface are of two types. The granular ones (high electron density) and the non-granular ones (low electron density). Also mitochondria, multivesicular bodies, golgi complex and granular endoplasmatic reticulum are observed. The cells establish contact by desmosomes which mainly characterize the surface of the top, appear and consist of differentiations of the plasma membrane (Castritsi, et al., 1978).

For the study of the effects of toxic solutions on the alimentary epithelium, Finasol OSR5 and the mixture Finasol OSR5/gas oil were used. Specimens were obtained from three categories of experiments. The procedure of embedding, fixation, sectioning and straining is described under the title "materials and methods". Specimens obtained from categories I, II, III of toxicity experiments were examined for possible alterations in shape, size and density of microvilli as well as the structure of cell organelles in comparison with the control (Fig. 7). Special emphasis was given to microvilli since it is the main structure of the epithelial cell which comes into direct contact with the external environment.

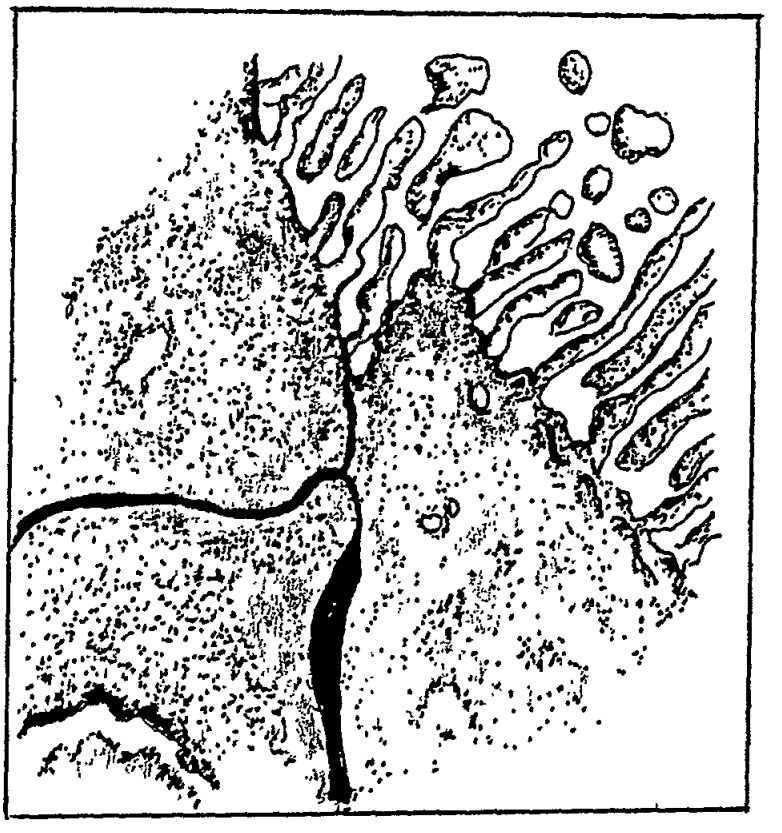

Fig. 7 Normal epithelial cells in the control

As is shown in Fig. 8, an alteration in shape, size and density of microvilli was observed. These alterations increase when the concentration of the toxic factor increases, while a total destruction is observed at lethal concentrations. In parallel, lysis of cell organelles, destruction of mitochondria, alterations in the dimensions of desmosomes and finally an increase of lysosomes and hyperoxidiosomes in number, are observed.

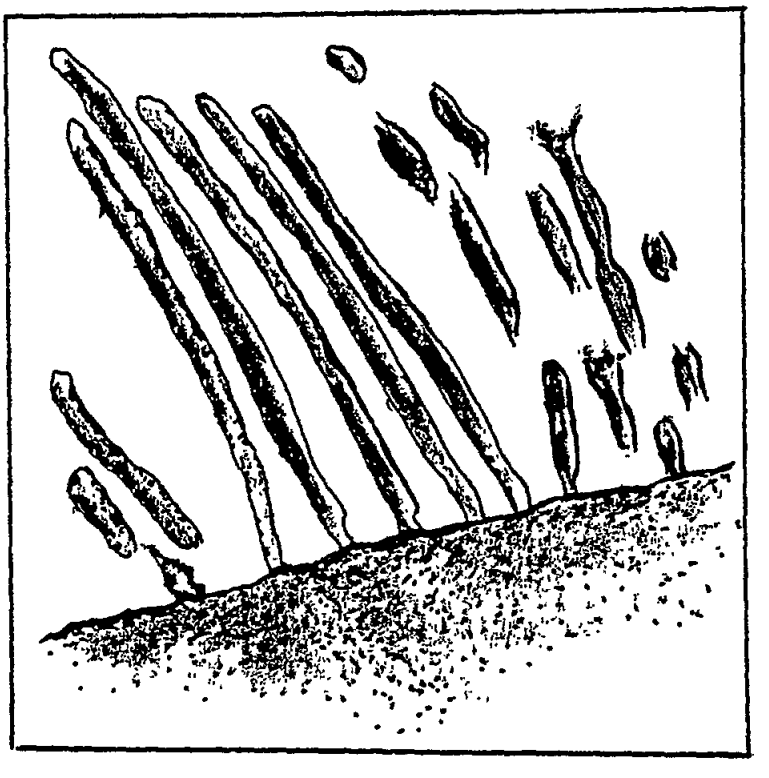

Fig. 8 Alterations in shape and density of microvilli

The expansion of the destruction is greater when the dispersant is used, rather than the mixture of dispersant-gas oil. This was expected however, since the mortality is greater (approximately double) in acute toxicity experiments. The completion of these experiments by means of electron microscopy requires repetitions and study of the effects of dispersants at very low concentrations and greater periods of exposure time of the experimental animals.

The further histochemical study of yolk platelets would be especially interesting.

Finally the alteration in desmosome dimensions must be further examined, taking into consideration their important role in the attachment of the cells and the normal function of epithelial tissues.

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ACUTE AND CHRONIC TOXICITY TO MARINE BIOTA OF WIDELY USED DISPERSANTS, PCBs, CHLORINATED PESTICIDES AND THEIR COMBINATIONS AND THEIR BIOMAGNIFICATION IN ALEXANDRIA REGION

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A B S T R A C T

The chlorinated insecticides DDT, endrin, lindane and toxaphene in a technical form were compared for their relative acute toxicity to Tilapia zilli and Gambusia affinis under standard laboratory conditions. Trichlorophenol was included as a common industrial waste chemical and also as one of the expected metabolites of some insecticides. DDT in a formulated form 25% E.C. was also used for comparison to investigate the impact of adjuvants on acute toxicity to fish. The fish were brought to the laboratory from two locations: the first was Mariut Lake in Alexandria lagoon where industrial and agricultural pollutants are discharged, mixed and diluted with drainage water; the second location was from Behera Governorate to the south of Alexandria, where agricultural pollutants are dominant in the agriculture drainage system.

According to the LC50 values, endrin was the most hazardous of the tested compounds for both species followed by DDT, toxaphene and then lindane. It was also found that formulated DDT (25% E.C.) was more toxic than the technical form for the two fish species. The results also indicated tha Gambusia affinis fish are more susceptible than Tilapia zilli and thus can be considered suitable for carrying out sensitive monitoring methods based on bioassays. Biomagnification tendency was highly demonstrated for DDT, followed by endrin, but not obvious for Lindane. Trichlorophenol was of very low toxicity. Organic mercuric compounds such as mercuric phenyl acetate was more toxic and liable for accumulation than mercuric chloride in Tilapia tissues.

1. INTRODUCTION

Hermanutz (1978) evaluated the acute and chronic toxicity of endrin and malathion to flagfish. The 96-hour LC_{50} for endrin was 0.85 $\mu g\ l^{-1}$ and the lowest concentration having a significant effect in the chronic toxicity was 0.30 $\mu g\ l^{-1}$. Mean accumulation factors for various life stages and exposure periods varied from 7100 to 15000. The 96-hour LC_{50} for malathion was 349 $\mu g\ l^{-1}$ and the lowest concentration having a significant effect in the chronic exposure was 11 $\mu g\ l^{-1}$.

Takeda (1978) studied the effects of DDT, BHC and PCBs on the growth of fish. Sublethal doses of BHC in food, inhibited growth of carp after two weeks of exposure.

Hansen (1980) found that in fish exposed for 9 days, lindane was taken up in proportion to its concentration in water levels between 10 and 70 $\mu g\ l^{-1}$.

Westernhagen et al. (1981) reported that chronic exposures of fish to bioaccumulative substances such as chlorinated hydrocarbons and heavy metals, have caused adverse effect on reproduction.

2. MATERIALS AND METHODS

Two strains of fry fish of Tilapia zilli were collected from El-Berdeesey fish farm at Alexandria Lagoon and from some agricultural drains at Etay-El-Baroud, Behera Governorate, 60 kilometres south of Alexandria. In addition, a field population of Gambusia affinis was collected from the Benera area. All three fish strains were kept in aquaria at the laboratory under standardized conditions of continuous aeration, temperature (25°C), and relative humidity of 70%. The mean length of Tilapia fry was 3.36 cm and of Gambusia 2.06 cm. The average body weight was 825 and 222 mg respectively. The fish were acclimatized under laboratory conditions for one week before the bioassay test. Standard imported fish diet was used in the aquaria cultures. The acute toxicity was carried out in six replicates for each concentration, in six glass vials, each containing 10 fish in aerated water.

Mortality counts were recorded after 24 and 96 hours. The mortality figures were corrected according to Abbott's equation depending upon natural mortality in the control replicates.

Tested Insecticides and Chemicals

The following chlorinated hydrocarbons were obtained in a pure technical form: p,p'-DDT, endrin, lindane and toxaphene. Trichlorophenol was included for comparison as a common industrial waste and one of the expected metabolites of some pesticides. DDT in 25% E.C. formulation was also used to indicate the role of formulating adjuvants on the acute toxicity of the active ingredient of the insecticide. Pure mercuric chloride was compared with phenyl mercuric acetate regarding their relative acute toxicity and rate of uptake by mosquito fish Gambusia affinis.

Accumulation of the tested compounds

The accumulation experiment was carried out using fry fish of Tilapia zilli of the Alexandria strain. Thirteen aquaria each containing 20 healthy fry fish were used for each treatment. The fish was exposed to one tenth of the 96-hours LC_{50} as a sublethal concentration. The exposure was continued for 28 days. Fish samples were taken for total content analysis of the incorporated insecticides. The accumulation experiment procedure was similar to the methodology applied and adopted by Davy and Kleerkoper (1972) and Jarvinen et al. (1977). The chlorinated insecticides were determined according to Thompson (1974).

3. RESULTS AND DISCUSSION

Acute toxicity of chlorinated hydrocarbons to Tilapia zilli and Gambusia affinis

Tables I and II include results of acute toxicity of Tech. endrin, DDT, lindane and toxaphene compared with 25% E.C. of DDT to Tilapia zilli fish from two separate locations, Behera Governorate and Alexandria Governorate. There was clear differentiation between the fish from the two governorates. The Behera population lives in agricultural drains and thus it is directly affected by residues of pesticides and other agricultural chemicals. Such continuous exposure might be responsible for build up of resistance to insecticides in such population especially when compared with the population less exposed like that in Mariut Lake in the vicinity of Alexandria where most of the effluents are diluted and mixed with organic loads from the municipal sewage and wastes. Table III shows folds of resistance in the Behera population.

Table I

Susceptibility of fry of Tilapia zilli, Behera strain to certain organochlorine pesticides.

Product	24-hour exposure			96-hour exposure		
	LC_{50} $\mu g\ l^{-1}$	Confidence Limits	Slope Values	LC_{50} $\mu g\ l^{-1}$	Confidence Limits	Slope Values
Endrin Tech.	24.34	16.70-35.47	1.79	10.09	7.56-13.46	2.33
P,P'-DDT Tech.	21.81	17.00-27.98	3.31	15.50	11.69-20.55	2.92
DDT 25% E.C.	12.78	9.56-17.08	2.32	9.52	7.36-12.31	3.20
Lindane Tech.	680.80	470.5-985.2	2.32	394.90	265.9-586.5	2.80
Toxaphene Tech.	88.27	59.64-130.6	1.71	68.82	45.95-103.1	1.66

Table II

Susceptibility of fry of Tilapia zilli, Alexandria strain to certain organochlorine pesticides.

Product	24-hour exposure			96-hour exposure		
	LC_{50} $\mu g\ l^{-1}$	Confidence Limits	Slope Values	LC_{50} $\mu g\ l^{-1}$	Confidence Limits	Slope Values
Endrin Tech.	1.20	0.85- 1.70	1.49	0.26	0.19- 0.35	2.72
P,P'-DDT Tech.	63.00	47.40-83.73	2.36	42.00	35.21-50.11	3.81
DDT 25% E.C.	27.50	23.19-32.62	3.42	9.50	8.02-11.25	3.45
Lindane Tech.	18.00	12.44-26.05	1.18	6.50	4.40- 9.32	1.39
Toxaphene Tech.	19.00	12.63-28.58	1.65	5.00	3.60- 6.95	2.05

Table III

Susceptibility of fry of Tilapia zilli (Alexandria and Behera strains) to certain organochlorine pesticides at 96-hours exposure period.

Product	LC_{50} µg l^{-1} (96 hours exposure)		Resistance ratio level (R.R.)
	Tilapia zilli strains		
	Alexandria	Behera	
Endrin Technical	0.26	10.09	38.81
p,p'-DDT Technical	42.00	15.50	00.37
DDT 25% E.C.	9.50	9.52	1.00
Lindane Technical	6.40	394.90	61.70
Toxaphene Technical	5.00	68.82	13.76

It was also observed that DDT in the E.C. form was higher in its acute toxicity to both strains. This indicates the importance of testing the formulated form for regulation of the pesticides.

Table IV presents the acute toxicity of the same chlorinated hydrocarbons to Gambusia affinis from Behera culture. In most cases Gambusia was more susceptible than Tilapia zilli, thus suggesting its use as a sensitive indicator for the hazardous levels of such pollutants.

Table IV

Susceptibility of fry of Gambusia affinis, Behera strain to certain organochlorine pesticides.

Product	24-hour exposure			96-hour exposure		
	LC_{50} µg l^{-1}	Confidence Limits	Slope Values	LC_{50} µg l^{-1}	Confidence Limits	Slope Values
Endrin Tech.	10.91	6.80-17.50	1.10	5.27	3.36- 8.27	1.16
P,P'-DDT Tech.	22.74	16.61-31.13	2.14	9.87	7.28-13.38	1.71
DDT 25% E.C.	58.58	43.15-79.52	2.69	27.69	21.32-35.96	1.99
Lindane TEch.	1129.00	811.5-1571	1.76	618.1	465.5-820.8	2.05
Toxaphene Tech.	71.20	49.16-103.1	1.82	49.48	35.29-69.37	1.99

The bioaccumulation rate of chlorinated hydrocarbon insecticides in Tilapia zilli strain

Table V and Fig.1 indicate the data of accumulation study of p,p'-DDT, endrin and lindane in Tilapia zilli of Alexandria strain. It can be observed that biomagnification was higher for DDT than endrin, while it was not clear for lindane.

This might reflect the relative rate of biodegraaation in the biological tissues in proportion to the retention affinity and storage of each of the tested compounds. It is well known that DDT has one of the highest partition coefficient values and thus its high lipid solubility will help in intensifying its biomagnification ability.

The present data are generally in agreement with those obtained by El-Bishry (1979) for DDT on Mugil cephalus and Tilapia rilotica. Tilapia species seem to be less susceptible than other fish species. Endrin was always more hazardous to fish than the other chlorinated hydrocarbons. Similar trends were recorded by Hermanutz (1978) and Anderson and De Foe (1980) for endrin on flag fish.

Table V

Accumulation of organochlorine pesticides in Tilapia zilli, Alexandria strain after exposure to sublethal concentrations (µg l^{-1})

Treatment			Time of exposure (days)				
			4	7	14	21	28
p,p'-DDT	1	p,p'-DDE	ND	ND	ND	ND	ND
		p,p'-DDD	ND	ND	220.78	545.46	1039.00
		p,p'-DDT	555.56	611.11	333.33	333.33	777.78
		Σ DDT[4]	555.56	611.11	554.11	878.79	1816.78
Endrin	2		327.44	167.44	297.64	446.50	595.35
Lindane	3		149.78	148.74	105.44	103.45	96.55

ND = Not detected
1 Sublethal concentration of p,p-DDT (3.57 ug l^{-1}
2 " " " Endrin (0.025 ug l^{-1})
3 " " " Lindane (0.631 ug l^{-1})
4 Σ DDT = p,p-DDD + p,p-DDT

Coats and O'Donnell-Jeffery (1979) and Randall (1979) reached the same conclusion that formulated insecticides are more toxic to fish and daphnia than the corresponding technical insecticides. This implies the requirement of submission of such data for the formulated compounds to assess their relative hazards to fish and non-target organisms in the environment. Similar results were obtained by El-Sebae et al. (1983).

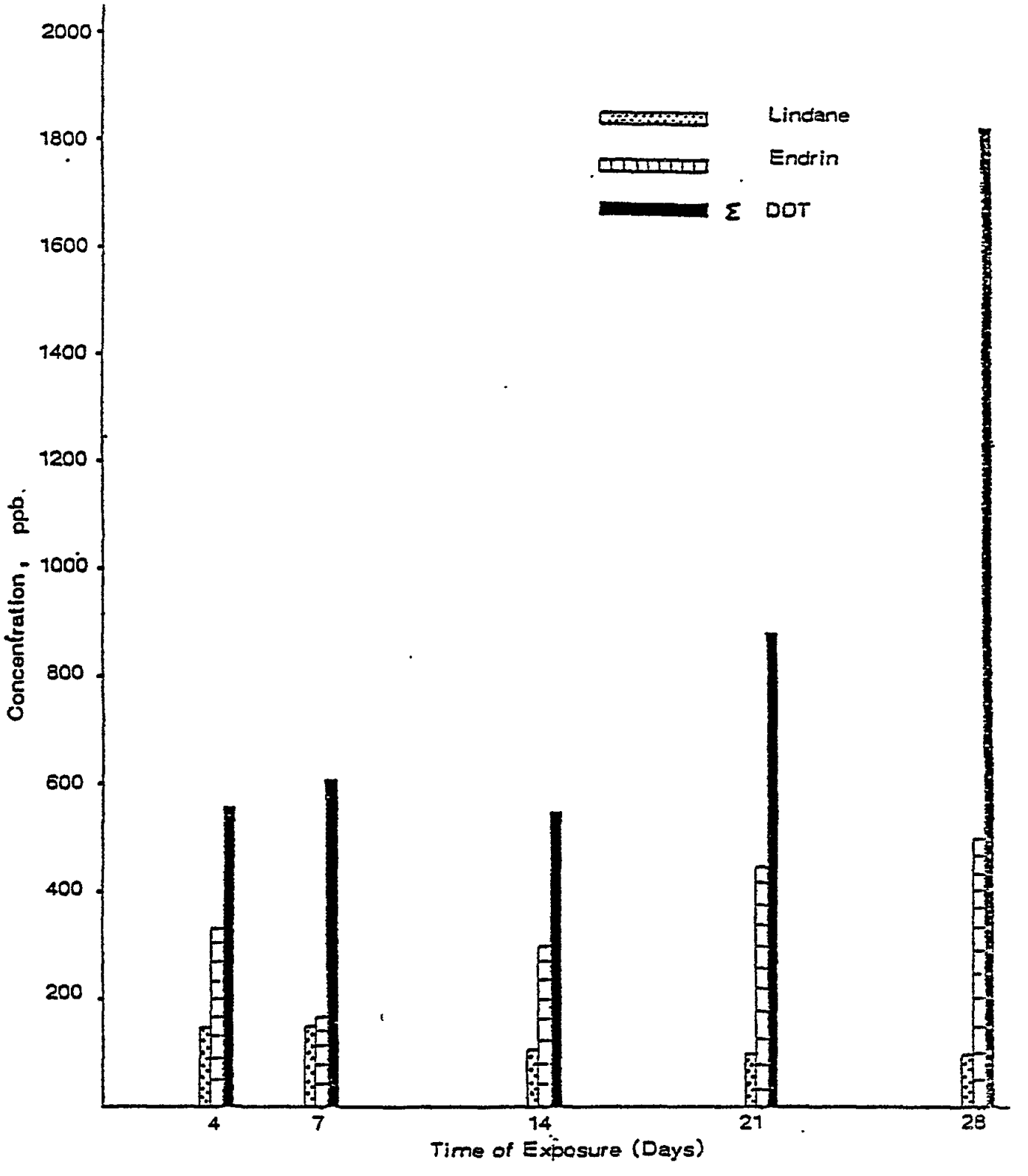

Fig.1 Accumulation of organochlorine pesticides in Tilapia zilli, Alexandria strain after exposure to sublethal concentrations (ug l^{-1})

Thus the chemical configuration of toxicant, the presence of adjuvants, the concentration, the time of exposure, the type of tested organism and the ecological environmental factors are all factors determining type and extent of the adverse effect to the non-target organisms, particularly the marine biota. The chlorinated hydrocarbons are the main abundant class in the land-based contaminants from the Egyptian coast of the Mediterranean Sea (El-Sebae and Abo-Elamayem, 1979).

Performance of mercuric compounds in aqueous ecosystem and their toxicities to Gambusia and Tilapia fish

Table VI presents the comparative data for the toxicity of mercuric chloride and phenyl mercuric acetate to Gambusia affinis after different time intervals. The results indicated the increased hazard of the organic mercuric compounds when compared with the inorganic ones.

The same trend was reshown in Table VII regarding acute toxicity to Tilapia sp.

Table VI

Toxicity of mercuric compounds to mosquito fish (Gambusia affinis)

Time interval (hrs.)	Mercuric chloride	Phenyl mercuric acetate
	24 hrs LC_{50} ppm	24 hrs LC_{50} ppm
4	No kill	0.040
24	1.2	0.026
48	0.95	0.024
72	0.80	0.020

Table VII

Toxicity and accumulation of mercury in Tilapia zilli (Alexandria strain)

Compound	24 hrs LC_{50} ppm	Accumulation after 24 hrs. % of initial concentration	
		heads	whole body
Hg cl_2	4.2	74%	18%
ph-Hg-acetate	0.3	74%	10%

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