MEDITERRANEAN ACTION PLAN
MED POL

UNITED NATIONS ENVIRONMENT PROGRAMME

WORLD HEALTH ORGANIZATION

EPIDEMIOLOGICAL STUDIES RELATED TO THE ENVIRONMENTAL QUALITY CRITERIA FOR BATHING WATERS, SHELLFISH-GROWING WATERS AND EDIBLE MARINE ORGANISMS

ÉTUDES ÉPIDÉMIOLOGIQUES RELATIVES À LA QUALITÉ DE L'ENVIRONNEMENT POUR LES EAUX SERVANT À LA BAIGNADE, À LA CULTURE DE COQUILLAGES ET À L'ÉLEVAGE D'AUTRES ORGANISMES MARINS COMESTIBLES

MAP Technical Reports Series No. 93

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This volume is the ninety third of the Mediterranean Action Plan Technical Reports 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, Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea, Environment Remote Sensing and Protection of Historic Sites.

Ce volume constitue le quatre vingt treizième 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, Centre régional méditerranéen pour l'intervention d'urgence contre la pollution marine accidentelle, Centre méditerranéen de télédétection et Protection des sites historiques.

PREFACE

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 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, exchange of information and assessment of the state of pollution and 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 inter-dependent 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.

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.

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.

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 substance listed in the annexes of the Protocol for the prevention of pollution of the Mediterranean Sea by dumping from ship 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);

- 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);

The Contracting Parties at their 6th Ordinary Meeting (Athens, October 1989) agreed to:

(a) Re-orient the research activities within MED POL in order to generate information which will also be useful for the technical implementation of the LBS protocol in addition to supporting monitoring activities;

(b) replace as from 1990 research activities A-L by the following five new research areas:

Research area I - Characterization and measurement

This area will include projects which cover the characterization (identification of chemical or microbiological components) and measurement development and testing of methodologies of specified contaminants;

Research area II - Transport and dispersion

This area will include projects which aim at improving the understanding of the physical, chemical and biological mechanisms that transport potential pollutants from their sources to their ultimate repositories. Typical topics will be atmospheric transport and deposition, water movements and mixing, transport of contaminants by sedimentation and their incorporation in biogeochemical cycles. Priority will be given to the provision of quantitative information ultimately useful for modelling the system and contributing to regional assessments;

Research area III - Effects

This area will include projects relevant to the effects of selected contaminants, listed in Annexes I and II of the LBS and Dumping protocols, to marine organisms, communities and ecosystems or man and human populations. Priority will be given to effects and techniques providing information useful for establishing environmental quality criteria;

Research area IV - Fates/Environmental transformation

This area will include projects studying the fate of contaminants (including microorganisms) in the marine environment such as persistence or survival, degradation, transformation, bioaccumulation etc. but excluding transport and dispersion which is dealt in area II;

Research area V - Prevention and control

This area will include projects dealing with the determination of the factors affecting the efficiency of waste treatment and disposal methods under specific local conditions as well as the development of environmental quality criteria and common measures for pollution abatement;

(c) define target contaminants or toher variables at periodic intervals depending on the progress of implementation of the LBS protocol;

(d) select project proposals on the basis of their intrinsic scientific validity, their Mediterranean specificity, and encourage whenever possible bilateral and multilateral projects among Mediterranean countries from the north and the south of the basin.

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.

This ninety third volume of the MAP Technical Reports Series contains final reports on six research projects completed within the framework of MED POL in Activity D - "Epidemiological studies related to the environmental quality criteria for bathing waters, shellfish-growing waters and edible marine organisms", which was in force at the time when the studies were carried out.

PREFACE

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 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 continue 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.

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;

- 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.

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:

- 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'evnironnement, 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.

Les sujets de recherche et d'étude inclus initialement dans MED POL Phase II étaient les suivants:

- 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);

- 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).

Les Parties contractantes au cours de leur sixième réunion ordinaire (Athènes, octobre 1989) ont convenu de:

(a) réorienter les activités de recherche menées dans le cadre du MED POL en sorte qu'elles engendrent des informations qui soient également utiles pour l'application technique du Protocole tellurique, en plus de l'appui apporté aux activités de surveillance continue;

(b) à compter de 1990, remplacer les activités A à L par les cinq nouveaux domaines de recherche ci-après:

Domaine de recherche I - Caractérisation et dosage

Ce domaine englobera des projets de recherche en matière de caractérisation (identification de constituants chimiques ou microbiologiques) et de dosage (mise au point et éssai de méthodes) de contaminants donnés;

Domaine de recherche II - Transfert et dispersion

Ce domaine englobera des projets visant à approfondir notre connaissance des mécanismes physiques, chimiques et biologiques qui véhiculent les polluants potentiels de leurs sources à leurs dépôts ultimes. Les sujets étudiés porteront notamment sur le transfert et le dépôt atmosphériques, les mouvements et le brassage des eaux, le transfert des contaminants par sédimentation et leur incorporation dans les cycles biogéochimiques. Priorité sera accordée à l'obtention de données quantitatives servant, en dernier ressort, à la modélisation des systèmes et à l'établissement des évaluations régionales;

Domaine de recherche III - Effets

Ce domaine englobera des projets relatifs aux effets de certains contaminants énumérés aux annexes I et II du Protocole tellurique et du Protocole relatif aux situations critiques: effets sur les organismes, les communautés et les écosystèmes marins, effets chez l'homme et parmi les populations humaines. Priorité sera accordée aux effets et techniques fournissant des données utiles pour établir les critères de qualité du milieu;

Domaine de recherche IV - Destinées/transformation dans l'environnement

Ce domaine englobera des projets portant sur l'étude de la destinée des polluants (micro-organismes y compris), dans le milieu marin, et notamment sur la persistance et la survie, la dégradation, la transformation et la bio-accumulation, etc., mais non sur le transfert et la dispersion qui sont traités dans le domaine II;

Domaine de recherche V - Prévention et lutte antipollution

Ce domaine englobera des projets traitant de la détermination des facteurs conditionnant l'efficacité des méthodes d'épuration et d'élimination des déchets sous des conditions locales spécifiques ainsi que de l'établissement de critères de qualité du milieu et de mesures communes de réduction de la pollution;

(c) définir des contaminants cibles ou d'autres variables à des intervalles périodiques en fonction de l'état de l'avancement de l'application du Protocole tellurique;

(d) choisir les propositions de projet sur la base de leur valeur scientifique intrinsèque, leur spécificité méditerranéenne et, chaque fois que possible, encourager les projets bilatéraux et multilatéraux entre les pays méditerranéens du nord et du sud du bassin.

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.

Ce quatre vingt treizième volume de la Série des rapports techniques du PAM comprend six rapports finaux exécutés dans le cadre de la Phase II du MED POL, dans l'Activité D - "études épidémiologiques relatives aux critères de la qualité de l'environnement pour les eaux servant à la baignade, à la culture de coquillages et à l'élevage d'autres organismes marins comestibles", activité en vigueur pendant la période où les études étaient effectuées.

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ECOLOGICAL AND MEDICAL ASPECTS OF JELLYFISH POISONING

by

Annamaria CARLI, Gian Luigi MARIOTTINI and Luigi PANE

ABSTRACT

The results pertinent to monitoring and to some epidemiological, biochemical and toxicological aspects concerning some jellyfish species found in the Ligurian Sea from 1987 to 1992 are reported here. Monitoring was carried out almost daily during the period 1987-89 along the whole Gulf of Genova with particular reference to Spotorno Bay (Savona). Further observations were carried out during 1990-92. On the whole, sighting normalization was everywhere noted after the bloom period. Epidemiological data, collected over a period of six years (1984-89) didn't show particular impact of jellyfish on humans. Dry weight, lipid, protein and fatty acid analyses were performed on some body structures of *Rhizostoma pulmo* and *Cotylorhiza tuberculata*. Oral arms and gonads have the highest dry weight, lipid and protein content. The gonads of both species contain the highest fatty acid percentages. Nevertheless, polyunsaturated fatty acids (PUFA) were abundantly found in the different body structures. *Rhizostoma pulmo* and the benthic sea-anemone *Anemonia sulcata* showed the highest cytotoxic effect on cultured fibroblasts; long-term growth inhibition was induced by toxin of *Aequorea aequorea*; *Pelagia noctiluca* showed low effectiveness.

1. INTRODUCTION

During past years, in several Mediterranean Countries many searchers have studied biology, ecology and morphology of jellyfish; nevertheless, owing to the low toxic characteristics of Mediterranean species, studies concerning jellyfish toxicity did not reach an interest for human pathology. In reality, jellyfish toxic properties are a worldwide health problem owing to the nematocyst sting, which causes irritative and/or toxic effects on human skin, as erythema accompanied by swelling, redness and vesicles as well as further dermonecrotic, neurotoxic and cardiotoxic complications in sensitive subjects. Fishermen and bathers are particularly exposed to these effects, extremely dangerous in some sea zones where species lethal also for humans occur. In consequence of the well-known problems caused by the bloom of Scyphomedusae that occurred during the last decades and affected nearly the whole Mediterranean, many Authors have concerned themselves with this phenomenon that, owing to the repercussions on touristic and fishing activities, involves both economic and health aspects.

The bloom started in the Eastern basin and in the Adriatic Sea and subsequently spread to the Western Mediterranean Sea; it similarly evolved everywhere, generally showing a remarkable proliferation from 1981 to 1984 and a decrease beginning from 1984-1985.

In the Eastern Mediterranean Sea, Piccinetti Manfrin and Piccinetti (1984) showed the frequency of *Pelagia noctiluca* in Egyptian and Greek waters during 1983; Dowidar (1984) reported the occurrence of *Pelagia noctiluca, Aurelia aurita* and other jellyfish species along the Egyptian coast line during summers of 1966, 1970 and 1982; Bingel (1991) and Bingel *et al.* (1991) studied jellyfish distribution along Turkish coasts; Lakkis (1991) recorded

proliferations of *Rhizostoma pulmo* during 1986 in Lebanese coastal waters. Recently, the occurrence of a new species coming from the Red Sea, namely *Rhopilema nomadica*, was noticed (Galil and Spanier, 1990); Galil *et al.*, 1990).

In the Adriatic Sea proliferations of *Pelagia noctiluca* were recorded along the Northern Croatian coast line (Benovic, 1984; Zavodnik, 1991). Furthermore, the distribution of *Pelagia noctiluca* and the seasonal variations in relation to changes of environmental factors were extensively studied (Rottini Sandrini and Stravisi, 1981; Piccinetti Manfrin and Piccinetti, 1983-84, 1984; Rottini Sandrini *et al.*, 1984; Piccinetti *et al.*, 1991).

Less dramatic consequences were observed in the Tyrrhenian and Ligurian Seas, despite remarkable alterations of the normal occurrence of Scyphomedusae were observed also in the Ligurian Sea (Bernard, 1984; 1991; Carli, 1991; Carli *et al.*, 1991a; Goy, 1984; Goy *et al.*, 1991; Balestra *et al.*, 1991); in some geographically receptive coastal zones sometimes they assumed the features of massive proliferation (Carli *et al.*, 1985), perhaps correlated both with meteorological and hydrological conditions and lunar cycle (Balestra *et al.*, 1991).

Piccinetti Manfrin and Piccinetti (1984) showed greater thickening of *Pelagia noctiluca* in the Sicily and Sardinia Channels than in Central-Southern Tyrrhenian Sea and in the Sardinia Sea.

Along the Maltese coastline, Axiak *et al.* (1991) recorded spatial and temporal distribution of *Pelagia noctiluca* during 1980-86, showing jellyfish thickening in coastal areas seemingly correlated with wind direction.

Some Authors (Goy, 1984; Vucetic, 1984) think that jellyfish bloom is a natural fluctuation of the population, nevertheless it has also been assumed that the jellyfish bloom is correlated with nutritional factors (Lakkis, 1991) or water circulation, particularly with the "intermediate Levantine" and perhaps the Atlantic surface current (Vucetic, 1984).

The correlation between Jellyfish proliferation and environmental factors (Lakkis, 1991; Zavodnik, 1991) or water pollution (Bingel, 1984; Wilkerson and Dugdale, 1984) was also suggested; nevertheless, Goy *et al.* (1991), by examining jellyfish distribution data from 1876 to 1986, denied pollution implications as giving rise to the bloom and hypothesized the appearance of cyclic phenomena correlated to modifications of the marine environment.

An interesting theory concerning the pollution influence is "hormesis" (Stebbing, 1991), which involves stimulation of growth processes by low concentrations of toxic agents; this phenomenon was observed in a number of experiments: e.g. stimulation of Hydroid reproduction in consequence of exposure to low concentrations of toxic substances was noted.

The bloom adaptive value, involving evolution processes connected to appearance of favourable mutations during rarity periods, followed by establishment during blooms was moreover suggested (Boero, 1991).

Furthermore, several researchers concerning biology, ecology and chemical aspects of some jellyfish species have been carried out in order to value their role in the marine ecosystems and food webs (Carli *et al.*, 1991b; Malej, 1991; Mastronicolis *et al.*, 1991).

The influence of jellyfish bloom on public health gave rise to epidemiologic studies, mostly in Adriatic areas, (Malej and Vukovic, 1984; Maretic, 1984; Maretic *et al.*, 1991) and in the Eastern Mediterranean Sea (Vlachos and Kontos, 1991), and also induced several Authors (Salleo *et al.*, 1983; 1984a; 1984b; 1986; 1991; Del Negro *et al.*, 1986; Avian *et al.*, 1991; Kem and Ostman, 1991; Ostman, 1991; Rottini Sandrini *et al.*, 1991) to the study of nematocysts, in order to improve the knowledge of morphology and toxic compounds, particularly of *Pelagia noctiluca*.

In this work, the results obtained by the Planktonology Section of the University of Genova during a three year (namely 1987-88, 1988-89 and 1991-92) research activity (W.H.O. Programme Med Pol Phase II), are reported. The research concerned the following subjects:

- carrying on of jellyfish monitoring in the Ligurian Sea and in other sea zones, and observation of the occurrence of larval stages (planulae and ephyrae) in plankton samples;
- completion of data concerning the energetic content of some jellyfish species, with particular reference to the fatty acid composition of jellyfish anatomic portions;
- study of some epidemiologic aspects of jellyfish proliferation;
- toxicologic properties of nematocyst venom of the macroplanktonic jellyfish *Aequorea aequorea, Pelagia noctiluca* and *Rhizostoma pulmo* and as comparison, of the benthic sea-anemone, *Anemonia sulcata* too, by estimation of cytotoxicity induced on mammalian cells in vitro.

The pertinent results are discussed here.

2. MATERIALS AND METHODS

2.1 Monitoring

Figure 1 shows the studied sea area, between Imperia and La Spezia. More detailed observations were carried out in Spotorno Bay (Savona), that from our previous researches (Carli *et al.* 1985; 1991a) resulted in a jellyfish aggregation area. The monitoring was performed as previously reported (Carli *et al.*, 1985; 1991a; Balestra *et al.*, 1991; Carli, 1991), during the period 1987-1989. At the same time meteorological (sky, wind) and sea conditions were recorded.

Further observations were performed during two oceanographic campaigns carried out on the oceanographic vessel "Minerva" (C.N.R.) in the Ligurian, Corsican and Northern Tyrrhenian Seas (August 1989) and in the Southern Tyrrhenian Sea in the neighbourhood of the Aeolian Archipelago (October 1990). Jellyfish sightings were recorded on a special card (Fig. 2a, b), handed over also to Public and Private Bodies of the Ligurian Riviera, showing a picture and a brief morphologic description of the most frequent jellyfish species (*Aurelia aurita, Pelagia noctiluca, Chrysaora hysoscella, Cotylorhiza tuberculata* and *Rhizostoma pulmo*). So, it was possible to obtain wide data concerning the phenomenon consistence and trend during the considered years and along the explored sea area.

Jellyfish monitoring was carried out also during the operations concerning another research programme (MED POL phase II, activity H) that studied the quality of neritic waters in two sea zones of the Gulf of Genova (Genova-Sturla, Riva Trigoso Bay) during the period 1988-91.

2.2 Plankton Examination

In order to examine the life cycle and to find the larval stages of Scyphomedusae, several plankton samples were collected in some sea zones. Pertinent results were compared with those recorded previously and simultaneously to the bloom of Scyphomedusae. The samples were collected by a plankton standard net WP2 and observed by stereoscopic microscope Zeiss SR and microscopes Zeiss Lab 16 and Leitz Diaplan (this one provided with brightfield/darkfield, phase contrast, colour video camera JVC TK 1085E and recording apparatus).

2.3 Jellyfish Collecting

The specimens utilized in biochemical and toxicological assays were collected in some sea areas of the Ligurian Sea (Camogli, Genova, Spotorno) and along the eastern Sardinian coasts (Porto Cervo) in the Northern Tyrrhenian Sea. In toxicological trials benthic Cnidarians, collected along the cliff of Sori (Genova) and the coast of Porto Cervo (Sardinia), were also used. Collected specimens were transferred to the laboratory taking care to avoid any damage and, when possible, they were maintained in an aquarium provided with mechanical and biological water filtration, at 18±2EC; otherwise they were kept at -20EC until utilization. Benthic sea-anemones were always and indefinitely maintained in an aquarium.

2.4 Wet Weight, Dry Weight and Protein Content

On different portions (bell, marginal bell, oral arms, gonads) of the jellyfish *Rhizostoma pulmo* and *Cotylorhiza tuberculata* collected in Spotorno Bay and in the sea area in front of Camogli (Genova), fresh weight, dry weight and some biochemical analyses were performed. The dry weight was valued by heating (60E C; 24h) and drying on silica gel (24 h). Weighings were valued by means of analytic balance (Mettler mod. H 20). Protein content was determined according to Lowry *et al.* (1951), by homogenizing in distilled water a portion of dried material, against standard of lyophilized bovine albumin (Merck), using a Philips Pye Unicam (mod.PU 8620) spectrophotometer.

2.5 Lipid Extraction

Lipid extraction was performed modifying the method of Folch *et al.* (1957). The samples were placed in a chloroform/methanol (2:1) mixture, homogenized, sonicated (5 min.) and afterwards left at room-temperature (2.5 hours). Later on they were filtered on paper previously washed with chloroform. Filtrates were placed in a separatory funnel with KCl 0.05M, in a 20% volume of the extraction mixture, shaken and maintained at 4E C for 12 hours to separate the two phases. Chloroformic phases were placed in a vacuum test-tube and concentrated to dryness. Extracts were dissolved with benzene and put in a previously weighed reaction test-tube (Supelco). After vacuum evaporation, test-tubes were weighed until a constant weight was obtained, to determine quantitatively the extract.

2.6 Fatty Acid Analysis

Fatty acids were analyzed, after methylation with BF_3 methanol (Supelco), by gas cromatograph Hewlett Packard Mod. 5830, provided with double column with FID, according to Metcalfe and Schmitz (1961).

2.7 Epidemiology

Epidemiological data, already published (Carli *et al.*, 1991a) and concerning exclusively stung children, refer to the period 1984-1989 and were kindly provided to us by the First Aid Station of the Children's Hospital "Istituto Giannina Gaslini" (Genova).

2.8 Toxin extraction

Some tentacles of reared in aquarium or just collected specimens were drawn and soaked in distilled water for 24 hours; later on, tentacles were disjointed by magnetic stirrer and homogenized by potter. Therefore, a suspension of almost entirely intact nematocysts mixed together to abundant tissue debris was obtained. The nematocysts were counted by means of hemocytometer (Thoma). Nematocyst discharge (70-80%) was induced by freezing-thawing cycles (-80E C/+37E C) and sonication in ice-bath. Finally, suspensions containing nematocyst toxin and several tissue factors, were successively pre-filtered on plankton nets from 200 to 20 µm and afterwards filtered with 0.45 µm sterile filters (Millipore Millex-HV) in order to carry out cytotoxicity assays on cell cultures.

2.9 Cell Cultures

Fibroblasts of the continuous cell line V79 (Chinese Hamster) have been maintained in DMEM medium (Biochrom) supplemented with 10% FCS (PAA Labor, Technogenetics), 1-glutamine, and antibiotics (penicillin 100 U/mR; streptomycin 100 µg/mR) (Biochrom). Cells have been maintained in CO_2 humidified incubator at 37E C in 25 cm^2 culture flasks (Costar), and handled upon vertical laminar flow; $1x10^6$ cells were used and treated in each experiment.

2.10 Toxicity Assays

The cytotoxic characteristics of venoms of the jellyfish *Aequorea aequorea, Pelagia noctiluca* and *Rhizostoma pulmo* and of the sea-anemone *Anemonia sulcata* have been tested. For each cnidarian species three different amounts of the toxic extract, in relation to the found nematocyst (N) number, were prepared and afterwards put in cell culture flasks, as follows:

DOSE A: 150,000 N/mR
DOSE B: 30,000 N/mR
DOSE C: 15,000 N/mR.

Furthermore, in order to compare the experimental data, a control test was prepared and two further doses of toxin of the sea-anemone *Anemonia sulcata* (DOSE D: 3,000 N/mR; DOSE E: 1,500 N/mR) were utilized.

In order to verify the action of venoms in time, cells were counted after 1, 2 and 3 hours of treatment, and after long-term treatment (five days).

Cell viability was verified by detaching from culture flasks, counting and dye exclusion (Trypan blue); growth rate was expressed as number of doublings, according to Patterson (1979).

3. RESULTS

The observation number performed in the Gulf of Genova during 1987 and 1988 are shown in Table 1. The observations were carried out almost daily, recording also the atmospheric and hydrologic conditions (sky, wind). Jellyfish sightings were always very scanty. Proliferation phenomena were not observed.

Table 1

Number of observations carried out in the Gulf of Genova during 1987 and 1988

Month / Year	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.
1987	-	-	-	24	17	26	30	31	30	23	28	31
1988	31	29	31	30	31	30	-	-	-	-	-	-

Table 2 shows the jellyfish abundance as a percentage of the observation total number carried out in Spotorno Bay (Savona) during the years 1987 and 1989. Because of difficulties met in evaluating accurately the number of observed specimens, we have chosen to distinguish sightings in abundance classes. In some cases abundant jellyfish occurrence can be noted, particularly ascribed to *Cotylorhiza tuberculata* and *Rhizostoma pulmo*. *Pelagia noctiluca* was less frequently observed.

During the period 1990-1992 sporadic sightings of *Cotylorhiza tuberculata, Rhizostoma pulmo* and *Pelagia noctiluca* occurred in the Riva Trigoso Bay, Camogli, Spotorno, and along the coast line of Genova.

Table 2

Jellyfish sightings as abundance classes during 1987 and 1989 in Spotorno Bay; percentages on total observation number.

Year	Month		J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.
1987	Observation number		-	21	9	-	18	30	31	30	30	19	30	14
	jellyfish abundance	*	-	100	100	-	100	100	90.3	83.3	73.3	100	100	100
		**	-	0	0	-	0	0	0	6.7	6.7	0	0	0
		***	-	0	0	-	0	0	9.7	10.0	20.0	0	0	0
1989	Observation number		15	25	12	-	4	19	30	30	29	31	15	6
	jellyfish abundance	*	93.3	92.0	100	-	75.0	94.7	90.0	90.0	100	93.6	93.3	100
		**	6.7	4	0	-	25.0	5.3	10.0	10.0	0	6.4	6.7	0
		***	0	4	0	-	0	0	0	0	0	0	0	0

* = absent; ** = scarce; *** = abundant

During the spring and early summer of 1992 proliferations of *Aequorea aequorea* (Leptomedusa showing bluish disk-shaped umbrella 20-25 cm in diameter, deep blue tentacles and pink-violet gonads), never observed during the bloom period, occurred in Riva Trigoso Bay and in the sea areas in front of Camogli and Genova; furthermore, considerable amounts of non-identified (because stranded or damaged) jellyfish were recorded in the sea zone in front of Genova during the spring of 1992.

The occurrence of planulae and ephyrae in plankton samples, collected in some stations and at various depths during the utmost bloom period, was recorded (Carli *et al.*, 1991a). On the contrary, in other samplings carried out in the sea areas in front of Varazze (Savona) and Camogli (Genova) before the bloom arose, neither planulae nor ephyrae were found (Bruzzone *et al.*, 1979; 1982). Also in several samples collected in some sea areas in front of Genova during 1987, 1988 and 1989 (Table 3), planulae and ephyrae were never recorded (Carli *et al.*, 1992; 1994), with the exception of the sporadic record of an Anthozoan planula during July 1989 in a neritic station (100 m depth).

Table 3

Plankton samples collected in two coastal stations (Genova-Sturla, Genova-Quinto) and in off-shore station of the Gulf of Genova during 1989, 1990 and 1991 in order to find cnidarian planulae and ephyrae

1989			1990			1991		
date	station	depth	date	station	depth	date	station	depth
17.05	GE-Sturla	20m	26.06	GE-Sturla	20m	11.04	GE-Sturla	20m
17.05	GE-Quinto	20m	26.06	off-shore	700m	11.04	off-shore	700m
17.05	off-shore	100m	02.08	GE-Sturla	20m	10.06	GE-Sturla	20m
26.06	GE-Sturla	20m	02.08	off-shore	700m	10.06	off-shore	700m
26.06	GE-Quinto	20m	25.09	GE-Sturla	20m	17.07	GE-Sturla	20m
26.06	off-shore	100m	25.09	off-shore	700m	17.07	off-shore	700m
18.07	GE-Sturla	20m	24.10	GE-Sturla	20m	01.08	GE-Sturla	20m
18.07	GE-Quinto	20m	24.10	off-shore	700m	01.08	off-shore	700m
18.07	off-shore	100m				09.10	GE-Sturla	20m
19.09	GE-Sturla	20m				09.10	off-shore	700m
19.09	GE-Quinto	20m						
19.09	off-shore	100m						

Dry weight, lipid, protein and fatty acid analyses were separately performed on some body structures (bell, marginal bell, oral arms, gonads) of *Rhizostoma pulmo* and *Cotylorhiza tuberculata*, in order to obtain a differential and detailed outlook of fatty acid composition.

In *Rhizostoma pulmo* dry weight resulted globally about 5% of the fresh weight; oral arms and gonads showed the highest values (5.19 and 5.13 respectively); in *Cotylorhiza tuberculata* dry weight appeared more variable, and widely superior to 5% of fresh weight. Highest values were found in gonads (8.10%).

On the whole, lipid percentage on dry weight was included between 0.7% (bell of both species) and 6.4% (oral arms of *C. tuberculata*).

Both in *Rhizostoma pulmo* and in *Cotylorhiza tuberculata* high protein content was found in oral arms and gonads.

Fatty acid percentage of lipidic extract varied from 16.3% to 55.3%. High fatty acid percentages were found in the gonads of both species. Polyunsaturated fatty acids were abundantly found (from 27% to 52% of total fatty acids) in the different body structures of *Rhizostoma pulmo* and *Cotylorhiza tuberculata*. The unsaturated/polyunsaturated ratio was included between 1.2 and 1.6; percent amount of essential fatty acids (linoleic and linolenic) resulted on the whole, included between 0.3% and 2%.

Table 4 reports epidemiologic data provided to us by the First Aid Station of the Children's Hospital "Istituto Giannina Gaslini" (Genova): on the whole, from 1984 to 1989, 20 children, generally being more at risk than adults due to not exercising enough care, were examined for jellyfish stings. All lesions turned out to be of minor significance, therefore none of them were hospitalized (Carli *et al.* 1991a).

Table 4

Epidemiologic data concerning stung children in the Ligurian Sea from 1984 to 1989

YEAR	cases (n)	age (mean)	seriousness	hospitalized
1984	5	14.6	slight	none
1985	0	-	-	-
1986	3	2.0	slight	none
1987	4	4.5	slight	none
1988	3	9.0	slight	none
1989	5	9.4	slight	none

Tables 5 and 6 show the results concerning toxicological assays carried out on fibroblast cell cultures.

During short-term assays (1 hour, 2 hours and 3 hours after treatment) toxins of *Rhizostoma pulmo* and *Anemonia sulcata* induced fast cell damage (Table 5): the highest dose produced a rapid decrease of cell viability also within 1 hour and killed nearly the whole cell population within two hours. The toxin of *Rhizostoma pulmo* acts more quickly and remarkably reduces viable cells within 1 hour. High toxic effects of dose B were evidenced in *Rhizostoma pulmo*, while in *Anemonia sulcata* the effects became evident only after two hours and furthermore, no substantial differences between two and three hours of treatment were noticed. The dose C produced slower effects in *Rhizostoma pulmo* and *Anemonia sulcata*. In *Aequorea aequorea* our data show scarce influence of dose B on cell viability (effects are evident only during the third hour) and a similar trend of doses A and C.

Long-term experiments (Table 6) showed high lethality of the toxins of *Anemonia sulcata* and *Rhizostoma pulmo* at highest doses. The toxin of *Anemonia sulcata* killed the whole cell population also utilizing the dose B.

The toxic effects of the venom of *Aequorea aequorea* were remarkable and the results were similar in all tested doses. Further observations need as regards the toxicity of *Pelagia noctiluca*, that showed lower effects on cell growth than those recorded using extracts of other species, probably due to the difficulties in nematocyst discharge.

On the whole cell doublings, calculated when viable cells were present at the end of the experiment, never reached positive values in *Aequorea aequorea*; they resulted in slow duplication activity in *Rhizostoma pulmo*, *Anemonia sulcata* and at the highest dose in *Pelagia noctiluca*, while they showed values closer to control test using dose C in *Pelagia noctiluca*.

Table 5

Short-term effects induced on V79 fibroblasts by the toxins of the jellyfish *Aequorea aequorea* and *Rhizostoma pulmo* and of the sea-anemone *Anemonia sulcata*

SPECIES	dose	plated cells	viable cells No. after treatment		
			1 hour	2 hours	3 hours
Aequorea	A	$1x10^6$	$9.5±0.7x10^5$	$6.0±2.1x10^5$	$5.0±1.4x10^5$
aequorea	B	$1x10^6$	$1x10^6$	$1x10^6$	$8.2±1.1x10^5$
	C	$1x10^6$	$9.5±0.7x10^5$	$6.5±3.5x10^5$	$6.5±3.5x10^5$
Rhizostoma	A	$1x10^6$	$2.5±0.7x10^4$	0	0
pulmo	B	$1x10^6$	$5.1±1.2x10^5$	$5.0±2.8x10^5$	$5.0±4.2x10^5$
	C	$1x10^6$	$1x10^6$	$6.8±3.8x10^5$	$6.1±5.5x10^5$
Anemonia	A	$1x10^6$	$5.5±1.4x10^5$	$1.0±2.0x10^5$	0
sulcata	B	$1x10^6$	$8.0±2.0x10^5$	$7.0±0.3x10^5$	$5.5±1.5x10^5$
	C	$1x10^6$	$9.5±0.5x10^5$	$9.0±0.5x10^5$	$8.0±2.0x10^5$

Table 6

Long-term effects induced on V79 fibroblasts by toxins of the jellyfish *Aequorea aequorea, Rhizostoma pulmo* and *Pelagia noctiluca* and of the sea-anemone *Anemonia sulcata*

SPECIES	dose	attachment	plated cells	viable cells final No	doublings
Aequorea aequorea	A	+/-	$1x10^6$	$4x10^5$	-1.32
	B	+/-	$1x10^6$	$5x10^5$	-1.00
	C	+/-	$1x10^6$	$6x10^5$	-0.74
Rhizostoma pulmo	A	0	$1x10^6$	0	-
	B	+	$1x10^6$	$2.5x10^6$	1.32
	C	++	$1x10^6$	$3.2x10^6$	1.68
Pelagia noctiluca	A	+	$1x10^6$	$2.5x10^6$	1.32
	B	++	$1x10^6$	$3.6x10^6$	1.85
	C	++	$1x10^6$	$4.4x10^6$	2.14
Anemonia sulcata	A	0	$1x10^6$	0	-
	B	0	$1x10^6$	0	-
	C	+	$1x10^6$	$1.8x10^6$	0.85
	D	+	$1x10^6$	$2.5x10^6$	1.32
	E	++	$1x10^6$	$2.9x10^6$	1.54
control	-	+++	$1x10^6$	$11.3x10^6$	3.51

4. DISCUSSION

The jellyfish bloom in the Mediterranean Sea was observed over several years in order to ascertain the distribution and thickening of Scyphomedusae.

In the Ligurian Sea the bloom assumed characters of abnormal proliferation, yet never reached levels recorded elsewhere. Our results confirm the normalization of this phenomenon during the years 1987-1992; the few proliferation which occurred along the surveyed area can be ascribed to normal fluctuations. Also examining the jellyfish life cycle, not many planulae and ephyrae were found in plankton samples collected before, during and after the bloom arose.

Several researchers provided different bloom explanations; in fact, the problem resolution is not easy. Nevertheless, it seems acceptable that neither hydrologic changes, as water circulation didn't show clear alterations during last decades, nor pollutant inputs, as blooms were also observed during far periods, supported jellyfish proliferation. Moreover, jellyfish decrease is not equally explainable.

Therefore, also owing to the disappearance, no hypothesis has explained the jellyfish bloom entirely; consequently in future it will not be possible to carry out sporadic observations in a lasting survey programme, but a continuous sighting plan will be necessary.

The same fatty acids found in Scyphomedusae by other Authors (Sipos and Ackman, 1968; Nakhel and Mastronicolis, 1984), were observed by our analyses. Consequently, lack of alterations in fatty acid content during blooms can be supposed, though specific data concerning the Ligurian Sea are not available. A constant unsaturated/polyunsaturated ratio was found in the various body structures of examined species (*Rhizostoma pulmo* and *Cotylorhiza tuberculata*). The variability in content of every fatty acid in the different structures of the same jellyfish can be related to their different functions; in particular, high fatty acid content can be observed in gonads of both species.

From a sanitary point of view, jellyfish in the Ligurian Sea didn't exert a peculiar influence on public health owing to the few and slight injuries recorded during the years 1984-1989.

Our toxicological studies showed evident cytotoxic effects and growth inhibition induced on fibroblast cell culture. Fast survival decrease and cytotoxicity was also noted during short-term experiments, using toxins of *Rhizostoma pulmo* and *Anemonia sulcata*. This is not surprising, considering the irritating properties of *Rhizostoma pulmo* and the well-known stinging characteristics of the sea-anemone *Anemonia sulcata*, that is able to produce serious effects also on humans (Maretic and Russel, 1982), as well as cytotoxicity in cultured cells (Mariottini *et al.*, 1993). On the contrary the scarce action of *Pelagia noctiluca*, that mainly supported the bloom in the Mediterranean and caused several problems to bathers and fishermen was noted. Such results require further research in order to understand how venoms act against cells. As suggested by others (Batista and Jezernik, 1992) nematocyst toxin can play a role in increasing the plasmalemma permeability of V79 cells by binding to various membrane phospholipids; then follows water uptake and, at high concentrations, rupture of external and also intracellular membranes. Furthermore, in such a case release of lysosomal enzymes, and generation of toxic oxygen radicals and activation of Ca-dependent phospholipases resulting in cell death, was suggested (Duvall and Willie, 1986). Also cell morphology can be changed by treatments with cnidarian toxins (Batista *et al.*, 1987; Batista and Jezernik, 1992).

Furthermore, mitochondrial alterations strengthened by Ca ions were noted in our other studies, utilizing the venom of *Anemonia sulcata*; this suggested also in this species the presence of toxins acting as phospholipases (Arillo *et al.*, 1994).

Dose increase dit not always produce an increase in mortality: this was noted in *Aequorea aequorea*; the significance of this phenomenon is not quite clear.

The toxin of *Aequorea aequorea* showed a slow effect, emphasized in long-term experiments, where it stopped cell growth at all doses.

Understandably, when toxins acted within three hours of treatment it killed the whole cell population rapidly; on the contrary a slow action affected cell proliferation and produced a negative growth rate.

As some researchers suggested that cnidarian toxicity comes both from nematocysts and surrounding tissues (Crone and Keen, 1969; Freeman and Turner, 1969; Endean and Noble, 1971; Wittle *et al.*, 1974; Mariscal, 1974), further studies, performed in our laboratory, could better elucidate the action mechanisms of venoms, by separating the toxic effects due to the nematocyst content and to tissue factors.

5. CONCLUSIONS

From the research results and from our previous studies (Balestra *et al.*, 1991; Carli, 1991, Carli *et al.*, 1985, 1991a) concerning jellyfish monitoring in the Ligurian Sea, the phenomenon can probably be the result of natural cyclic fluctuations, already described in several species, perhaps correlated though not in a determinant way, with environmental or trophic factors as pointed out by other Authors (Goy, 1984; Vucetic, 1984; Goy *et al.*, 1991; Lakkis, 1991; Zavodnik, 1991).

The comparison between the fatty acid content approach during (Carli *et al.*, 1991b) and after the bloom can confirm lack of alteration in lipid content during the proliferation and therefore it is not possible to hypothesize implication of food quantity and quality in supporting the phenomenon.

The toxicological studies performed on cell cultures, which we consider an efficient alternative method to the utilization of living organisms, showed a considerable cytotoxic effect and cell growth decrease. Further studies need to explain the action mechanisms of jellyfish toxins in damaging biomembranes and in altering membrane permeability, as the implications on public health are evident.

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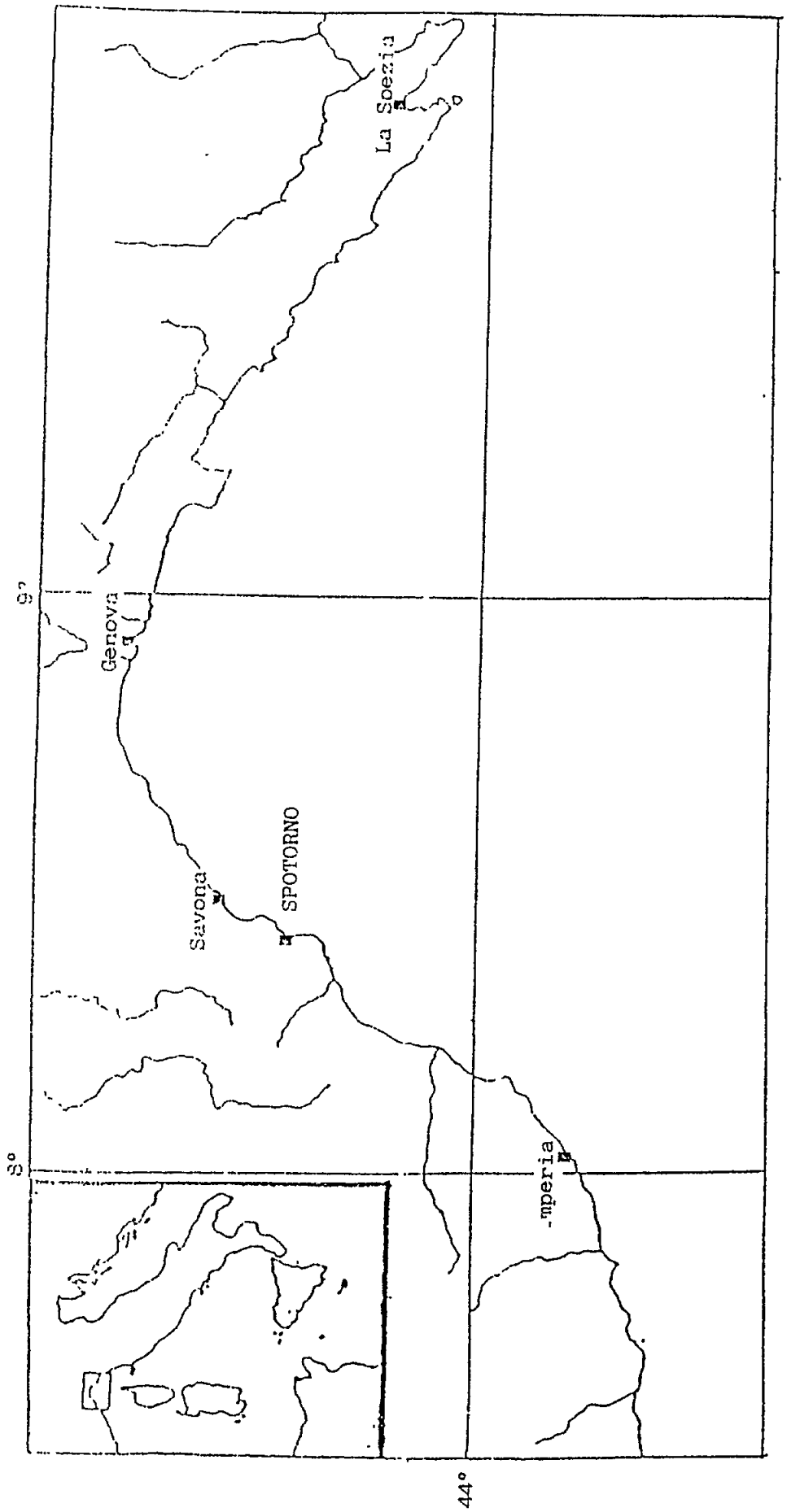

Figure 1. Ligurian Sea: examined sea area.

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JELLYFISH PROJECT MEDITERRANEAN SEA

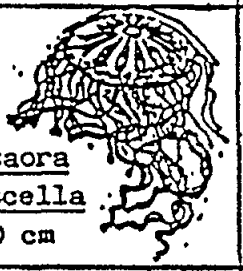
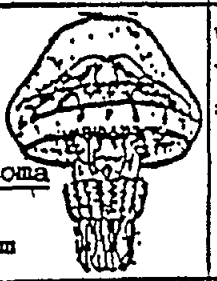
features of main species of the Ligurian Sea	COLOUR	BELL	MARGINAL BELL	ORAL ARMS
A  Aurelia aurita 30–50 cm	whitish, with violet or pink marginal bell and oral arms	flattened, with violet-pink edges; 4 half-moon-shaped gonads	8 lobes edged with several tentacles	4 oral arms with fringings in the lower portion
B  Pelagia noctiluca 6–8 cm	transparent, light pink, violet spots, phosphorescent	hemispherical lengthened	edge divided into 16 lobes; 8 tentacles	4 oral arms
C  Chrysaora hysoscella 20–40 cm	yellowish with brown-violet lines on bell surface	hemispherical/ flattened	32 lobes 24 tentacles	4, very long, with several fringings in the upper part and scallop-edged in the lower part
D  Cotylorhiza tuberculata 25–35 cm	brown or yellowish	flattened with raised central portion	14 main lobes subdivided in several small lobes	8, with several violet or purple mouths
E  Rhizostoma pulmo 15–60 cm	whitish, with violet borders and lobes	hemispherical bell-shaped	several lobes; lack of tentacles	8, divided in the lower part hundreds of small mouths half-way nearly
other species				

Figure 2a. Front page of the jellyfish sighting card.

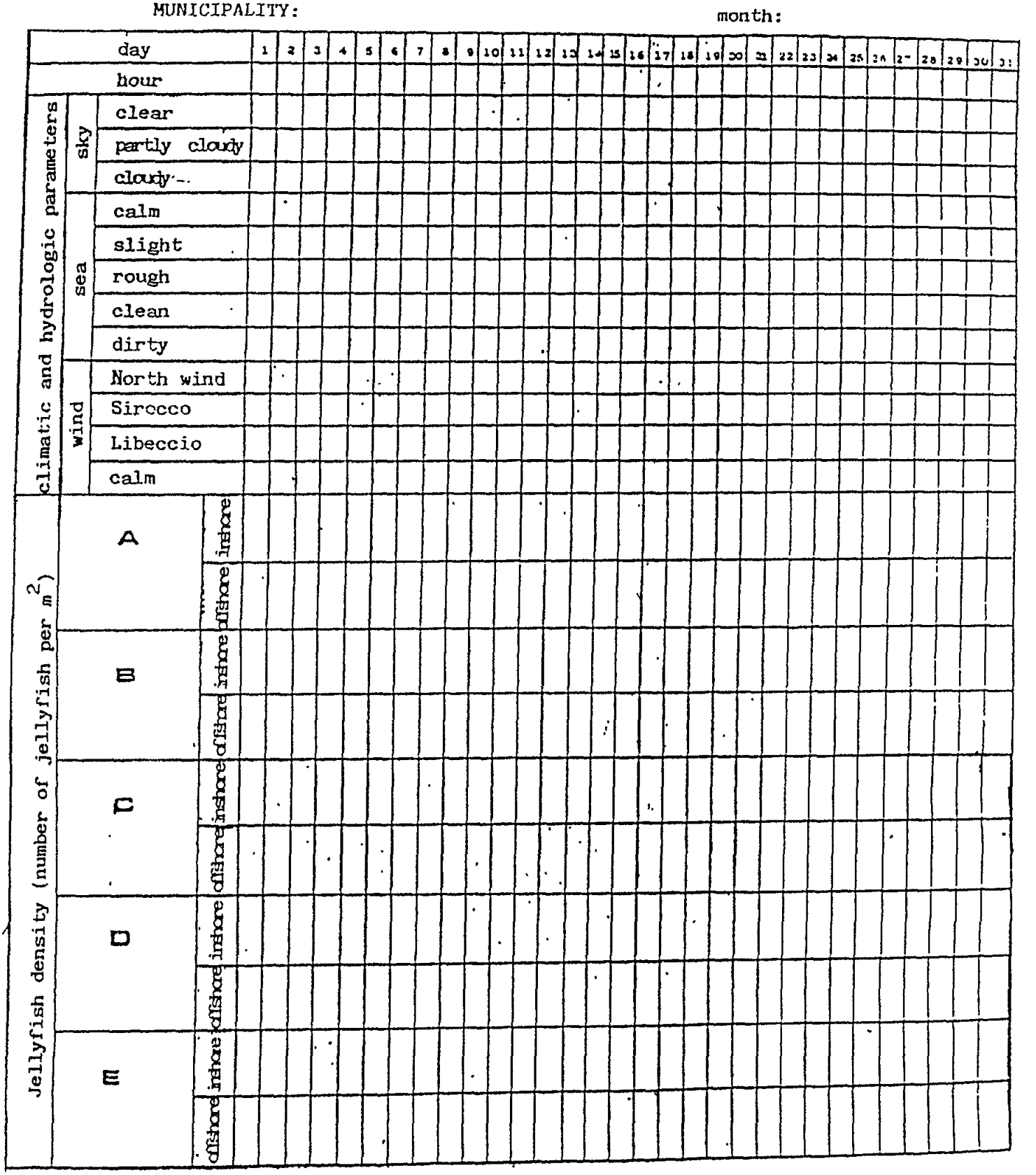


Figure 2b. Back page of the jellyfish sighting card.

EFFECT OF BATHING ON HUMAN SKIN FLORA

by

M. Papapetropoulou and S. Sotiracopoulou

ABSTRACT

In the present study the microbial skin flora of healthy volunteer bathers before and after bathing in Southern Greece coastal areas was monitored.

For quantitative microbial dermal analysis the pad technique was used.

The microorganisms determined on the skin before and after bathing and in seawater were Faecal coliforms (FC) *Escherichia Coli* (EC), *Faecal Streptococci* (FS), *Pseudomonas aeruginosa Staphylococci* coagulase positive and negative Total fungi *Candida albicans* and *Aeromonas hydrophila*.

Apart from the above microbiological parameters Aerobic bacteria count (SPC) per cm^2 of the skin were calculated before and after bathing.

Irrespective of the quality of bathing water, not a single bather had any colonization of FC, FS, *P. aeruginosa* and *A. hydrophila* on their skin.

Total fungi counts on the skin of bathers showed a statistically significant increase (p 0019) after bathing in polluted beaches.

Staphylococcus species and Aerobic bacterial count varied before and after bathing. However the quality of water had no obvious effect on this variation.

1. INTRODUCTION

Normal human skin serves as a barrier between the external environment and the internal milieu, and is colonized by two types of microbial flora; those grown on the skin which are relatively stable in number and composition are called resident flora; these organisms include staphylococcus epidermidis, various micrococci, anaerobic and aerobic diptheroides. The above microbial flora is involved in pathogenic processes (Price 1938). In contrast a second group of organisms, called transient flora is derived from exogenous sources intermittently colonizing the skin and do not normally grow there. These include *staphylococcus aureus,* streptococci, various enteric organisms and fungi. Under the proper circumstances the latter organisms may become pathogenic and develop foci of infection (Melish 1983). The number and kind of bacteria present on skin vary with the site cultured, sampling and culture methods utilized, presence of moisture, nutrients, pH and body temperature. (Aly and Mailbach 1977). Skin bacteria live naturally in the outer keratinized layers of the epidermis (Kligman 1965) and consequently bathing in seawater will remove many of them (Price 1938). This mechanical removal process allows a proportion of the surface bacteria to be spread into t he seawater.

The main type of human exposure to pathogenic microorganisms in the marine environment is through direct contact with polluted seawater. Mujeriego's study (1982) conducted in Spain claimed that the highest morbidity rate observed in bathers (2%), concerned skin infection.

Bathing has been associated with a number of diseases or disorders affecting the skin (UNEP/WHO/IAEA, 1988) (Yoshpe - Purer and S. Golderman 1987). The causative agents are *S. aureus*, *P. aeruginoa, C. albicans*, etc., which may be found in water.

The purpose of the present study was to monitor the microbial skin flora of healthy bathers before and after bathing in Southern Greece coastal areas and to find (1) the possible colonization of the skin from microorganisms present in coastal waters and (2) changes of dermal flora after bathing.

2. MATERIAL AND METHODS

The project was carried out over a period of three years (1989-91). The collection of skin samples was performed during peak hours on weekends during the bathing season (May - October). The number of volunteer bathers included in this study was 262.

The following organisms were determined on the skin before and after bathing. Faecal coliforms (FC), *Escherichia Coli* (EC), *Faecal Streptococci* (FS), *Pseudomonas aeruginosa (P. aeruginosa)*, Staphylococci coagulase positive (Staph+) and negative (Staph-), Total fungi (T), *Candida albicans* (*C. albicans*) and *Aeromonas hydrophila (A. hydrophila)*.

On the day of skin sampling we established the presence of the above microorganisms in the bathing water.

Apart from the above microbiological parameters, Aerobic Bacteria Count (SPC) per cm^2 of the skin was calculated before and after bathing.

2.1 Skin sampling

Sterilized square pads of moquette 4x4 (Holt 1966) backed with heavy aluminium foil were used. The sampling was carried out using four different pads, all of which were stamped on the skin of the hairless forearm surface just before bathing without lateral movement. After removal the pads were evenly pressed on the culture media. The pads were plated on mannitol salt agar (Oxoid), Mc Conkey agar, Cook Rose Bengal agar (Difco) and Plat Count agar (Oxoid). McConkey agar and mannitol salt agar were incubated at 35E C, Cook Rose Bengal agar at 25E C for five days and Plat Count agar at 22E C for 48h.

Colonies were counted and the result was given as mean colony count per cm^2. After bathing each individual would stand approximately five minutes for drying without dabbing and then the moquette cultures were repeated.

Attention was paid to stamp the same points of the forearm.

The beaches studied are shown in Figure 1.

Staphylococcus species were identified by gram strained and catalase, coagulase and DNASE tests. The identification of fungi was based on colonial morphology and gram strain.

2.2 Water sampling

Seawater samples from twenty beaches were collected during the bathing activity of the volunteer bathers according to the UNEP/WHO guidelines (1982).

Seawater was tested using standard methods (APHA 1989) for the following parameters. FC, EC., FS, *P. aeruginosa,* Staphylococci(+) and (-), TF, *C. albicans* and *A. hydrophila*.

2.3 Microbial analysis of seawater

All monitoring was performed with the following media, incubation temperatures and techniques: for TC and FC Lauryl tryptose broth (double and single strength (Oxoid) incubated at 35±0E C for 48 h with Most Probable Number (MPN) technique. TC and FC confirmation were performed according to the standard method techniques (APHA 1989); for FS Slanetz and Bartley medium (Oxoid) incubated at 42E C ± 0.5E C for 48 h with MF technique; FS were verified by catalase production, growth in brain heart infusion broth at 44.5E C within 48 h and growth in bile broth medium after 72 h at 35E C; for *P. aeruginosa* Centrimide agar (Oxoid) incubated at 42E C for 48 h with MF technique. Typical and atypical colonies were verified by using Milk agar; for *S. aureus* m Staphylococcus medium (Difco) incubated at 35E C for 24 h with MF technique. Three to five colonies were picked, gram stained (typical microscopic morphology) and confirmed with catalase, coagulase and DNAse tests; for *A. hydrophila* Glutaminate - Starch - Penicillin (GSP) (Merk) agar with an elevation of pH at 8 and incorporation of ampicillin 10 mg/R to the original formula were incubated at 30E C for 48 h with MF technique. *A. hydrophila* were identified by API 20 NE system (Analytical Profile Index) (La Balme les Grottes 38390 Montalieu - Vercieu, France) using the modification of the diluent proposed by Mac Donell *et al.* (1982) for estuarine samples. For total fungi and *C. albicans* Cooke Rose Bengal agar (Difco Laboratories) incubated at 25EC for 5 days with MF technique. The identification of fungi and *C. albicans* was based on colonial morphology and gram stain.

2.4 Statistical analysis

t-test and analysis of covariance were used for the statistical analysis of the study.

3. RESULTS

Table 1 shows the occurrence of enteric and non-enteric parameters studied in coastal water during the bathing activity of 262 volunteers bathers. From the twenty two beaches studied eight were considered polluted.

Bathers' skin was not colonized by FC, FS, *P. aeruginosa* and *A. hydrophila*, in spite of the presence of these microorganisms in seawater.

S. aureus and *C. albicans* were not isolated from either coastal waters or skin samples.

Table 1

Occurrence of enteric and non-enteric parameters in coastal waters

YEAR	BEACH	FC	FS	*P. AERUGINOSA*	STAPH COAGULASE (+)	STAPH COAGULASE (-)	*T. FUNGI*	*C. ALBICANS*	*A. HYDROPHILA*
1988	1. KAMINIA BEACH	7	10	>200	<1	<1	10	<1	<1
"	2. ARAHOVITICA BEACH	<1	15	5	<1	<1	20	<1	<1
"	3. ACTEON BEACH	<1	2	<1	<1	<1	60	<1	<1
"	4. AGIA BEACH	9	18	<1	<1	<1	10	<1	<1
"	5. PROASTION BEACH	<1	<1	<1	<1	<1	20	<1	<1
"	6. PSATHOPYRGOS BEACH	>2400	18	5	<1	<1	2	<1	>100
"	7. RIO BEACH	7	<1	<1	<1	<1	<1	<1	<1
"	8. SELIANITICA BEACH	<1	<1	<1	<1	<1	<1	<1	<1
"	9. ALISOS BEACH	<1	<1	<1	<1	<1	40	<1	<1
"	10. PARALIA BEACH	>2400	32	4	3	14	40	<1	<1
"	11. MONODENTRI BEACH	<1	<1	<1	<1	<1	<1	<1	<1
"	12. LAKOPETRA BEACH	<1	<1	<1	<1	<1	40	<1	
1989	1. RIO BEACH	0	<1	<1	<1	<1	1-	<1	<1
"	2. AG. BASILIOS BEACH	25	5	3	<1	<1	40	<1	<1
"	3. LOGOS BEACH	>2400	200	<1	<1	<1	<1	<1	<1
"	4. E.O.T. BEACH	30	50	<1	<1	<1	<1	<1	<1
"	5. VRAHNEIKA BEACH	240	10	<1	<1	<1	80	<1	<1
"	6. AGIA BEACH	1100	250	100	<1	300	100	<1	<1
"	7. TSOUKALEIKA BEACH	150	10	<1	<1	<1	<1	<1	<1
"	8. LAUBIRI BEACH	<1	30	<1	<1	<1	<1	<1	<1
"	9. RODODAFNI BEACH	<1	<1	20	<1	<1	<1	<1	<1
"	10. NIKOLEIKA BEACH	<1	<1	5	<1	<1	10	<1	<
1990	1. ACHAI BEACH	50	<1	<1	<1	2	6	<1	<1
"	2. PSATHOPYRGOS BEACH	1000	15	<1	<1	5	10	<1	<1
"	3. ROITIKA BEACH	1000	2	<1	<1	30	8	<1	<1
"	4. RIO BEACH	100	<1	<1	<1	<1	10	<1	<1
"	5. E.O.T. BEACH	1000	15	<1	<1	<1	50	<1	<1
"	6. KAMINIA BEACH	100	5	500	<1	<1	10	<1	<1
"	7. RODINI BEACH	<1	5	<1	<1	30	10	<1	<1
"	8. AGIA BEACH	>2400	300	<1	<1	20	10	<1	<1

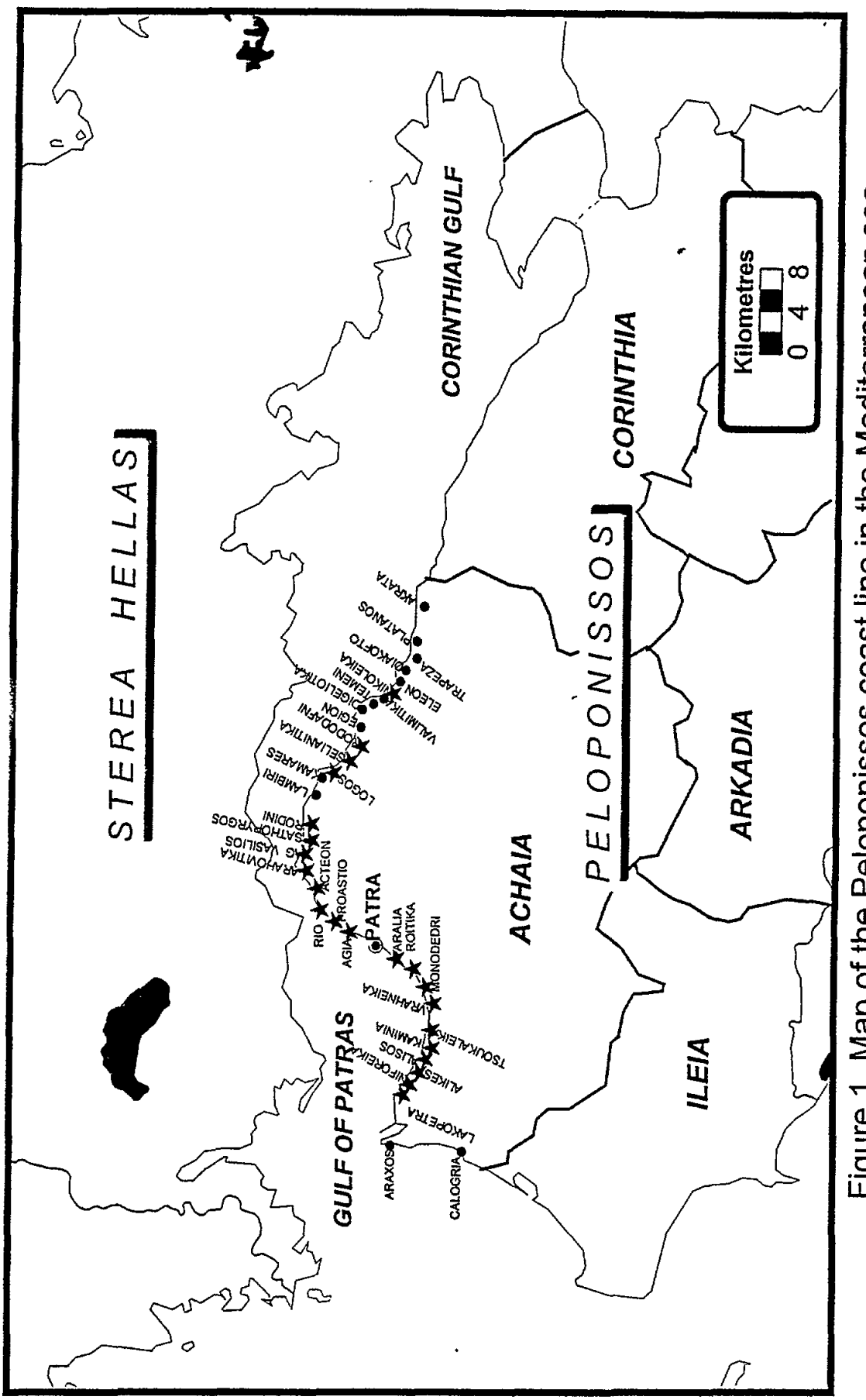


Figure 1. Map of the Peloponissos coast line in the Mediterranean sea.
★ Beaches studied

Statistical analysis concerning the increase in skin colonization for staphylococcus spp. T and Total count bacteria before and after bathing in polluted and unpolluted beaches revealed a statistically significant increase for T after bathing in polluted coastal waters (p 0.019), while it was not the case for staphylococcus spp and Total bacteria count (p. 061 and p:0.81 respectively).

When we examined whether the changes in SPC depended on a) SPC before bathing and b) beach (analysis of covariance) we found a statistically significant difference between beaches F: 1.81, p: 0.025 but not depending on level of pollution (F: 0.01, p: 0,91).

The statistical analysis of the data shows also that larger initial values of SPC tended to be associated with smaller changes in SPC after bathing.

Table 2

% bathers who showed variation in microorganisms
in polluted and unpolluted beaches

	SPC	STAPH	TF
Unpolluted beaches	64/198 = 32.31	49/198 = 25.41	38/198 = 19.21
Polluted beaches	19/64 = 29.71	19/64 = 29.72	22/64 = 34.4
Comparison	x^2_1 = 0.06 p = 0.81	0.26 0.61	5.48 0.019

Polluted beaches $ 1000 TF/100 mR seawater
SPC : Aerobic Count Bacteria
Staph: Staphylococcus spp
TF : Total fungi

4. DISCUSSION

Over the last few years there have been an increasing number of skin complaints among bathers in relatively "unpolluted beaches". Thus, it is of paramount importance to determine(a) quantitatively selected microorganisms on the skin before and after bathing, and (b) the presence of these microorganisms in the bathing water during the test period.

The composition of the normal flora varies depending on body location. The upper arms are relatively dry and have lower bacterial counts.

In this study we used the pad culture technique for skin forearm sampling.

The results could have been different if different culture techniques had been used. For example: colony count obtained by the scrub method showed a marked decrease immediately after bathing. (Williamson and Klligman 1965).

Holt (1970) claimed that aerobic bacterial counts decrease slightly immediately after bathing and slowly returned to the prewash numbers in 24 h.

Statistical analysis of our data shows that larger initial values of aerobic bacterial counts (SPC) tended to be associated with smaller changes in SPC after bathing although not statistically significant. That means that in high SPC population of skin there is small mechanical washout into the water.

Staphylococcus species are potential pathogens associated with skin problems in humans. The origin of these organisms in seawater is attributed to human activity. *Staphylococcus* species being salt-tolerant can survive in the marine environment (UNEP/WHO/IAEA 1988).

S. aureus was not isolated from the coastal waters studied. However its presence in the same beaches was confirmed in a previous study (manuscript in preparation).

Staphylococcus coagulase negative (particularly *S. epidermids*) counts on the skin of the bathers before and after bathing varied irrelevant with the actual number of these organisms in bathing water and the microbial pollution of the beaches.

P. aeruginosa was isolated in rather high densities ($ 100 CFU/100 mR of water) in 3 (13,6%) out of 22 beaches studied, while in another 6 (27,2%) the densities were very low (# 20 CFU/mR). However, despite its presence in seawater, *P. aeruginosa* does not colonize healthy bather's skin.

Recently there has been increasing evidence that *P. aeruginosa* is implicated in skin infection through bathing in contaminated seawater (UNEP/WHO/IAEA 1988). Our study does not support this hypothesis, at least not for bathers with healthy skin.

A number of fungal species are pathogenic to man. In seawater more than 16 species of fungi have been isolated (Izquiero *et al.* 1986). Other studies tended to isolate fungal pathogenic species in seawater (Bernard *et al.* 1988).

Recent studies in Greece (Papadakis *et al.* 1990) resulted in isolation of Aspergillus and Fusarium species in seawater.

In our study, although total fungi counts on the skin of bathers after bathing are irrelevant when compared to the actual number of these organisms in seawater, there is a statistically significant increase on the skin of bathers in the polluted beaches (p:0.019). This means that there is an increase probability of fungal dermatitis for bathers in polluted coastal waters.

No bathers were found to be colonized with FC irrespective of the quality of bathing water. It is known that enterobacteriaceae, when exposed to an unfavourable, oligotrophic environment, undergo some structural and metabolic changes in order to survive. For example, when *E. coli* is exposed to seawater loses its adhering capacity on the uroepitherial cells

probably because of damage of their fibrial (Munro *et al.* 1987) as all manose sensitive hemagglutination disappeared in the presence of seawater. The above lost property could be a probable explanation for the absence of colonization of enterobacteriaceae on the skin.

Results from this study suggest that the skin flora of the bathers varied before and after bathing. However, the quality of the water had no obvious effect on this variation except for Total fungi where there is a statistically significant increase on the skin of bathers in polluted beaches.

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DERMAL DISEASE IN BATHERS IN MARINE COASTAL AREAS

by

PAVLOS VLACHOS and PARASKEVAS KONTOES

1. INTRODUCTION

The Mediterranean covers only 1% of the world's sea surface, while the population of its shores is now in excess of 100 million, and a further 100 million tourists arrive each year. It has been estimated that it takes nearly 80 years for its water to be renewed. Extensive analyses carried out over the last decade have shown that considerable areas of the Mediterranean are polluted by sewage, industrial wastewater and oil spills.

Several epidemiological studies were performed in order to investigate the relationship between the microbial quality of coastal seawater and morbidity among bathers. In many of the above studies, an excess of various diseases was found among swimmers over non-swimmers, regardless of the microbial quality of the water. Another potential source of infection is the spray from polluted seawater containing pathogenic bacteria and viruses, which may penetrate the respiratory system (Baylor *et al.*, 1977). Since in bathing water the entire body is exposed to infection, illnesses associated with the eye, ear, skin and upper respiratory tract may be even more common than gastrointestinal disorders, as observed by Stevenson (1953) and Mujeriego *et al.* (1982).

Pollution of the seas with microbial or chemical pollutants is a daily fact. Since this pollution exists in areas around Athens, which are sometimes used for swimming, the question is raised as to whether this could be the cause of dermal diseases. This is supported by the high frequency of several dermal diseases, especially during the summer (e.g. fungal diseases etc.).

There is an impression that seawater is a probable reason for dermal diseases especially if there is a high content of pollutants, and very often several dermal diseases are diagnosed during the summer purely by chance. On the other hand high exposure to the sun for tanning purposes, which is not attributable to the seawater, causes mild or severe dermal diseases (erythema, sun burns etc.). Jellyfish stings or insect bites also cause dermal problems. In order to find out if dermal diseases are caused under such circumstances we carried out the following project.

2. SCOPE OF THE STUDY

As mentioned above, there is an impression that seawater is a probable reason for dermal diseases especially if there is a high content of pollutants.

The objectives of the present study were to determine the frequency of dermal diseases among swimmers and non-swimmers and to prove if there was any correlation between dermal diseases and seawater or other agents of coastal areas.

3. MATERIAL AND METHODS

Material and methods of the study include three sections:

3.1 Collection of dermal disease cases referred to the Poison Control Centre for a period of two years (July 1987 - June 1989). Here, we have to notice that we evaluate cases which are referred to the Poison Control Centre on the possibility and suspicion that the dermal problem is related to a toxic factor mainly of the environment (seawater, sand soil etc.). Only in those cases has an evaluation been done in accordance with the possibility of reasons due to seawater.

3.2 Determination of incidences of dermal diseases in a wide range of sea swimmers by means of a questionnaire, in comparison with an equal number of individuals with no seawater contact or other kind of coastal activities.

Studies were conducted during two bathing seasons (June-September 1987 and June-September 1988).

The study took place during 1987 in the Attica area and during 1988 in the area of Thira island (Santorini), Cyclades.

3.2.1 Study of Attica Area (1987)

3.2.1.1 Beaches evaluation study

Some of the beaches in the Attica area are considered as being contaminated mainly due to bacteriological analysis of seawater samples. Those areas have been characterized as being contaminated because analytical data have shown a faecal coliform concentration of more than 500 per 100 mℝ of seawater. Swimming is prohibited in these areas although they are used by the public from time to time.

3.2.1.2 Participation on the study

Participation in the study was carried out by completion of special questionnaires (see appendix No I) after interviews conducted.

3.2.1.3 Number and characteristics of sample

For the above purpose a sample population consisting of 600 individuals was used and answers to specific questionnaires were collected.

The main characteristics and conditions of collection for all samples were as follows:

Sex: 324 males (54%) and 276 females (46%)
Age: varying from 16 to 72 years
Profession or occupation was not considered
Samples were taken from the broad Attica area.
The study was carried out from June to September 1987.

The above material was divided into three groups under certain criteria as described in each group. An effort was made to have three groups with an equal number of individuals (200) irrespective of sex or age.

Group 1: The main characteristic of these individuals was that they were not bathers during the summer of 1987. Also they did not have increased exposure to sun apart from the usual. They lived in the northeastern suburbs of Attica.

Group 2: The main characteristic of these individuals was that they were summer bathers during the period June-September at least 10 times or more in coastal areas of Attica. The areas were well maintained and allowed for swimming (Saronic and Evoic bay). Exposure to the sun was either short or long following swimming.

Group 3: Same characteristics as above, the only difference being that they were swimming in areas polluted by microbial or chemical factors. They were also exposed to the sun for short or long periods after swimming.

The incidence of dermal diseases was examined during the swimming period and for 20 days following the end of it. "Dermal disease" is considered to be any dermal reaction i.e. eruption, itching, discoloration of skin, with or without symptoms which was evaluated by the physician. However, erythema due to sun or sun-burn and any kind of lesion, i.e. insect bites or jellyfish stings were not included.

3.2.2 Study of Thira Area (1988)

The beaches of the island are considered to be contaminant free and there is no limitation for using them.

3.2.2.1 Participation to study

Participation in the study was carried out by completion of special questionnaires (see appendix No II) after interviews conducted.

3.2.2.2 Number and characteristics of sample

For the above purpose a sample population consisting of 740 individuals was used and answers to specific questionnaires were collected. The questionnaire is included. All the above individuals lived on the island of Thira (Santorini) for, at least, a period of two months.

The above material was divided into two groups under certain criteria as described in each group. An effort was made to have two groups with an equal number of individuals (370) who did not differ significantly as regards sex and age.

Group 1: The main characteristic of these individuals was that they were not bathers during the summer period of 1988. They also did not have any increased exposure to the sun apart from the usual.

Other characteristics were:
Sex: 180 males and 190 females
Age: varying from 16-78 years
Profession was not considered

Samples were taken from beaches all over the island
The study was carried out from June to September 1988.

Group 2: The main characteristic of these individuals was that they were summer bathers during the period June-September at least 10 times and more in coastal areas of the island. The areas were well maintained and allowed for swimming. They also had exposure to the sun for short or long periods after swimming.

Other characteristics were:
Sex: 188 males and 182 females
Age: varying from 16-70 years
Profession was not considered
Samples were taken from beaches all over the island
The study was carried out from June to September 1988.

3.3 Compilation of records of cases of dermal diseases among bathers in polluted and non-polluted coastal areas, through cooperation with medical centres in Greece

This part of the study examines dermatological cases referred to General Hospitals especially Dermatological Departments. The cases were noted in individuals who had contact with sea and sand suspect factors. Details of the procedure followed is given below:

3.3.1 The total number of dermal cases from two General Hospitals of Attica during 1987-1988 was evaluated. The frequency between the summer (swimming) season and winter (non-swimming) season was also compared in order to check the possible increase of cases relating to each season.

3.3.2 Two hundred cases from Dermatological Hospitals in Athens, ranging in age from 12 to 70 years, during the summer months (June-October) of 1987-1988 were studied and evaluated. Chronic dermatological problems were not considered.

The study examined the relation between dermal problems and swimming. Two hundred dermal patients were asked if they had swum in the sea 10 days prior to developing a problem. No relevance was given to whether the water was contaminated or not.

4. RESULTS

4.1 In this section the cases of dermal diseases referred to the Poison Control Centre were analyzed.

During 1987-1988, 193 cases of dermal diseases ranging in age from 10 to 75 years were referred to the Poison Control Centre. Most of them concerned contact dermatitis (123). Eighteen of them were from jellyfish stings, fourteen were from possible insect bites and the rest for no special reason. It is characteristic that the rate of cases between summer (June-September) and winter (October-May) was almost the same (48,7%/51,3%).

From the 94 cases during the summer season, only 42 (44,7%) were from swimmers, while 52 (55,3%) had no relation to the sea. Ten cases concerned dermatitis developed after contact with oil or tar on the sand (table 1).

Table 1

Dermatological cases referred to Poison Control Centre
in two years 1987-1988

Cases in		Swimmers	Non-swimmers
Swimmer season	94	42(44,78%)	52(55,3%)
Other seasons	99		
Total	193		

4.2 In this section the incidence of dermal diseases was determined in Attica.

The incidence of dermal diseases was examined during the swimming period and for 20 days thereafter. "Dermal disease" is considered to be any dermal reaction i.e. eruption, itching, discoloration, with or without symptoms which was evaluated by the physician. However, erythema due to sun or sun-burn even any kind of lesion, insect bites or jellyfish stings were avoided.

Dermal diseases observed in the three groups were as follows: (table 2, fig. 1,2).

It can be seen that there is a very slight difference in the frequency of dermal disease in the three groups. No significant difference in dermal diseases were found between non-swimmers and swimmers in polluted or non-polluted seawater (P>0,05).

4.3 The incidence of dermal diseases was examined during the swimming period and for 20 days thereafter. "Dermal disease" is considered any dermal reaction i.e. eruption, itching, discoloration, with or without symptoms which was evaluated by the physician. However, erythema due to sun or sun-burn even any kind of lesion, insect bites or jellyfish stings were avoided.

Dermal diseases observed in the two groups were as follows: (table 2, fig. 3,4).

Table 2

The incidence of dermal diseases in Attica

Dermal disease	Number of cases in groups		
	1	2	3
Impetigo	1	2	1
Herpes simplex	1	1	1
Scabies	-	1	-
Folliculites	1	1	2
Eczematoid dermatitis	2	1	2
Pityriasis	3	2	3
Favus	-	1	-
Other	2	3	3
	10	12	12
Incidence %	5	6	6

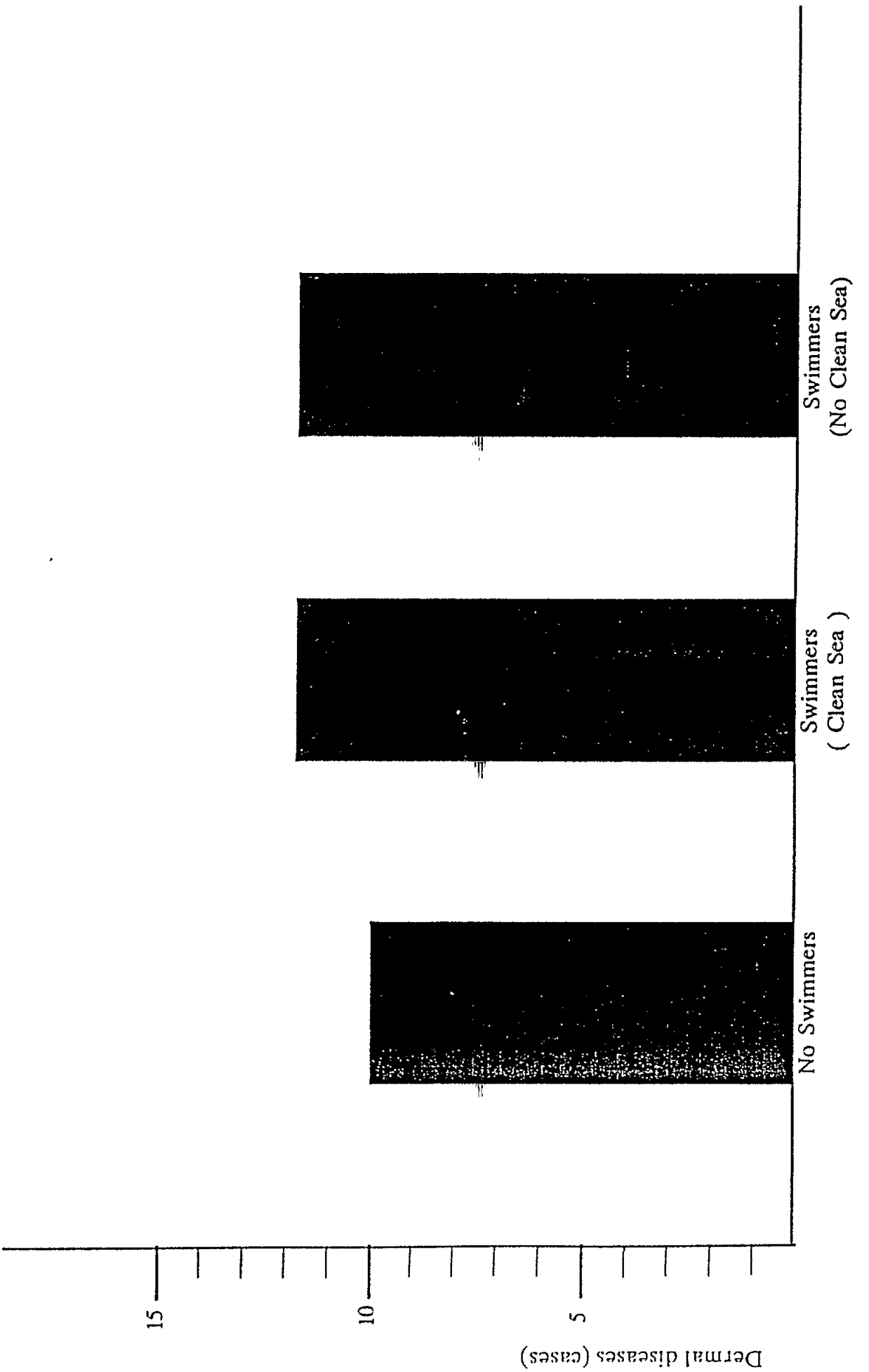

Figure 1. Distribution of dermal diseases in three groups of Attica

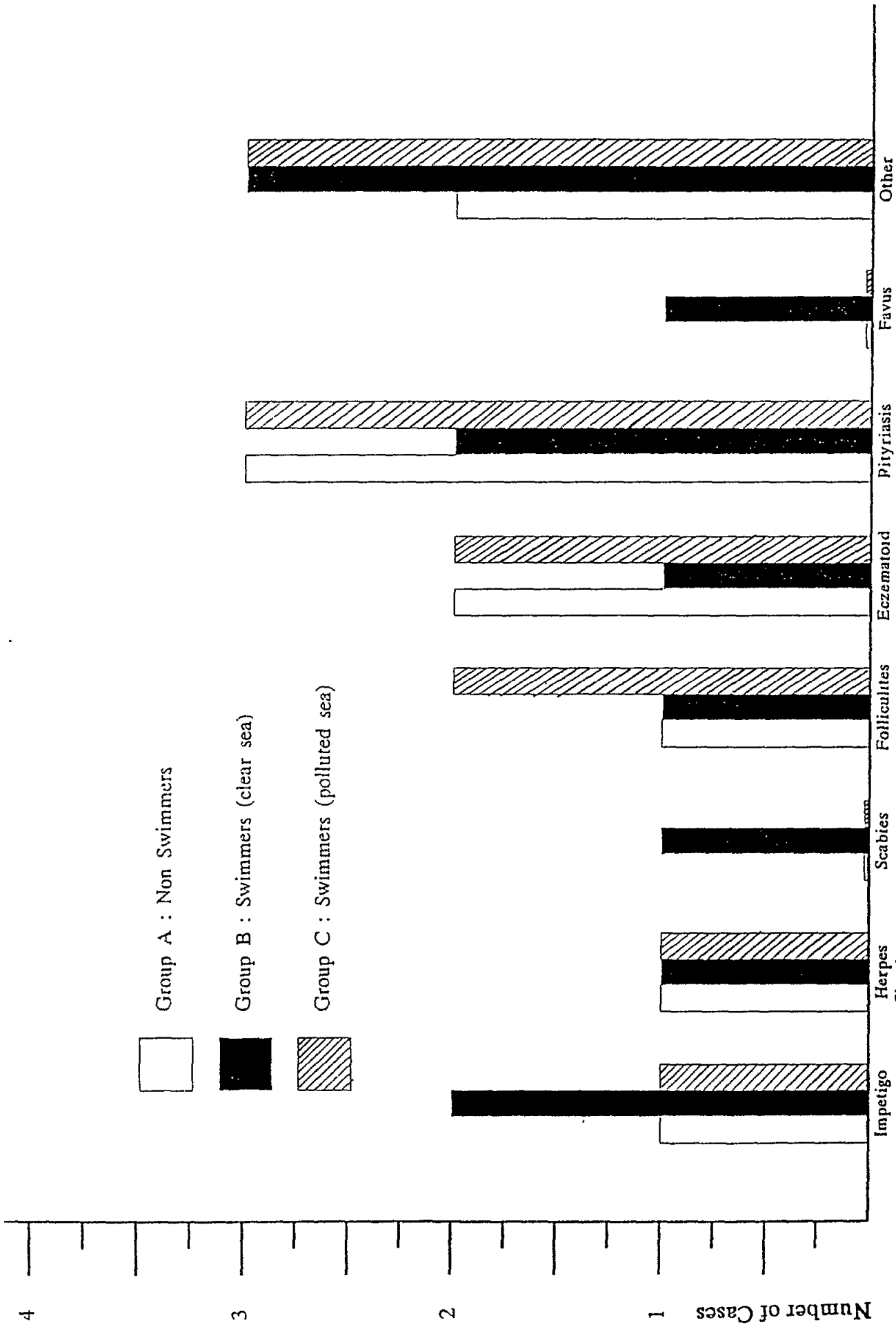

Figure 2. Distribution of dermal diseases in three groups of Attica

Table 3

The incidence of dermal diseases in Thira island

Dermal disease	Number of cases in groups	
	1	2
Impetigo	3	3
Herpes simples	1	2
Scabies	-	2
Folliculitis	2	3
Eczematoid dermatitis	2	2
Pityriasis	4	3
Favus	-	1
Other	4	4
	16	20
Incidence %	4,3	5,4

It can be seen that there is a very slight difference in the frequency of dermal disease in the three groups. No significant difference in dermal diseases were found between non-swimmers and swimmers in polluted or non-polluted seawater (P>0,05).

4.3 In this section the results of dermatological data from General or Dermatological Hospitals were analyzed.

4.3.1 250 dermal diseases in individuals from 15 to 78 years of age during the years 1987-1988 were analyzed in accordance with data obtained from two General Hospitals.

The monthly average was 21,3 cases for the non-bathing period (October-April) and 20,5 cases for the bathing period (May-September) (table 4).

Table 4

The monthly average of dermatological cases in two General Hospitals

Total cases in years 1987-88	Monthly average of cases in bathing and non bathing period	
250	20,5	21,3

It can be seen that there was no difference in the monthly average of dermal disease in the swimming and non-swimming periods.

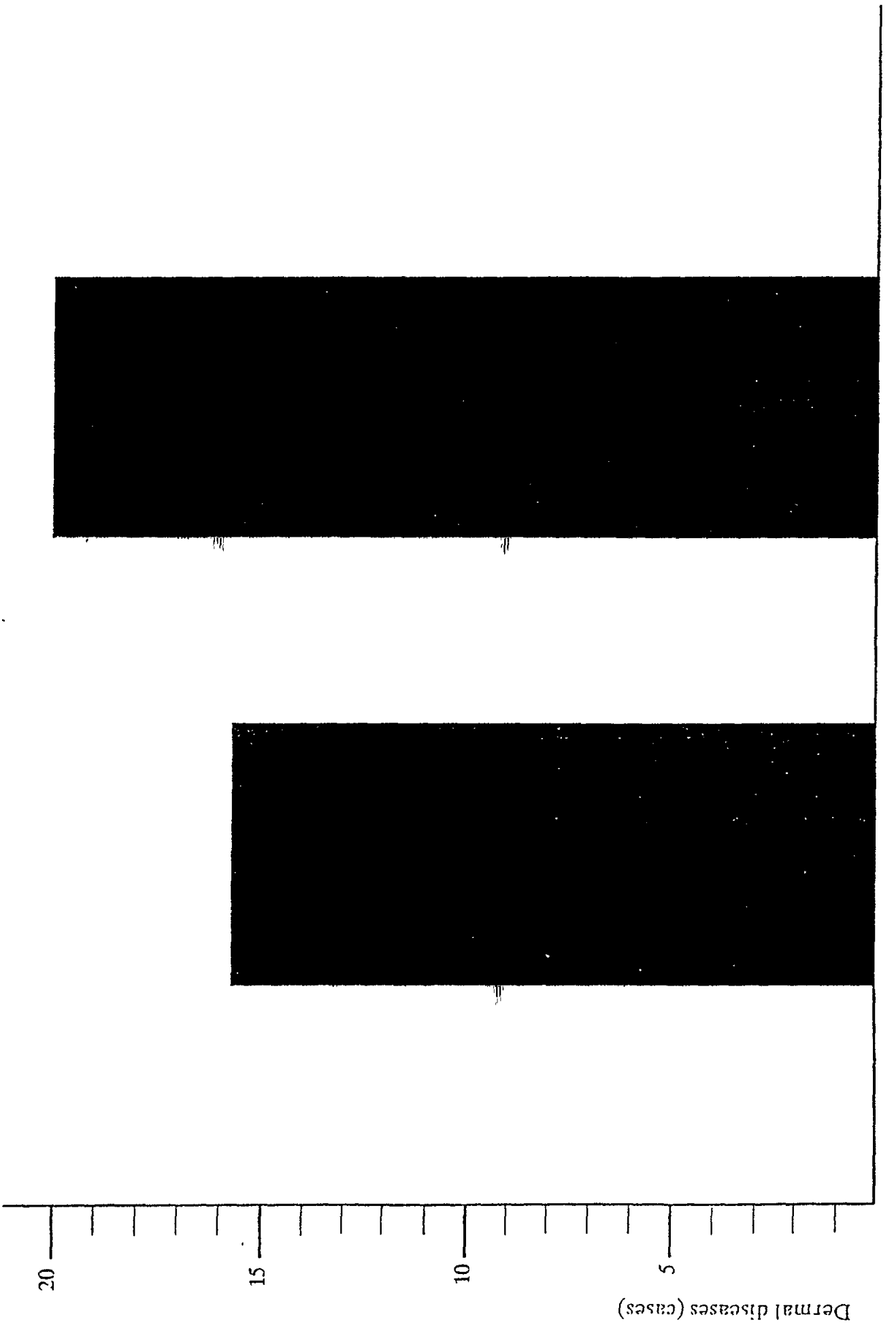

Figure 3. Distribution of dermal diseases in two groups of Thira

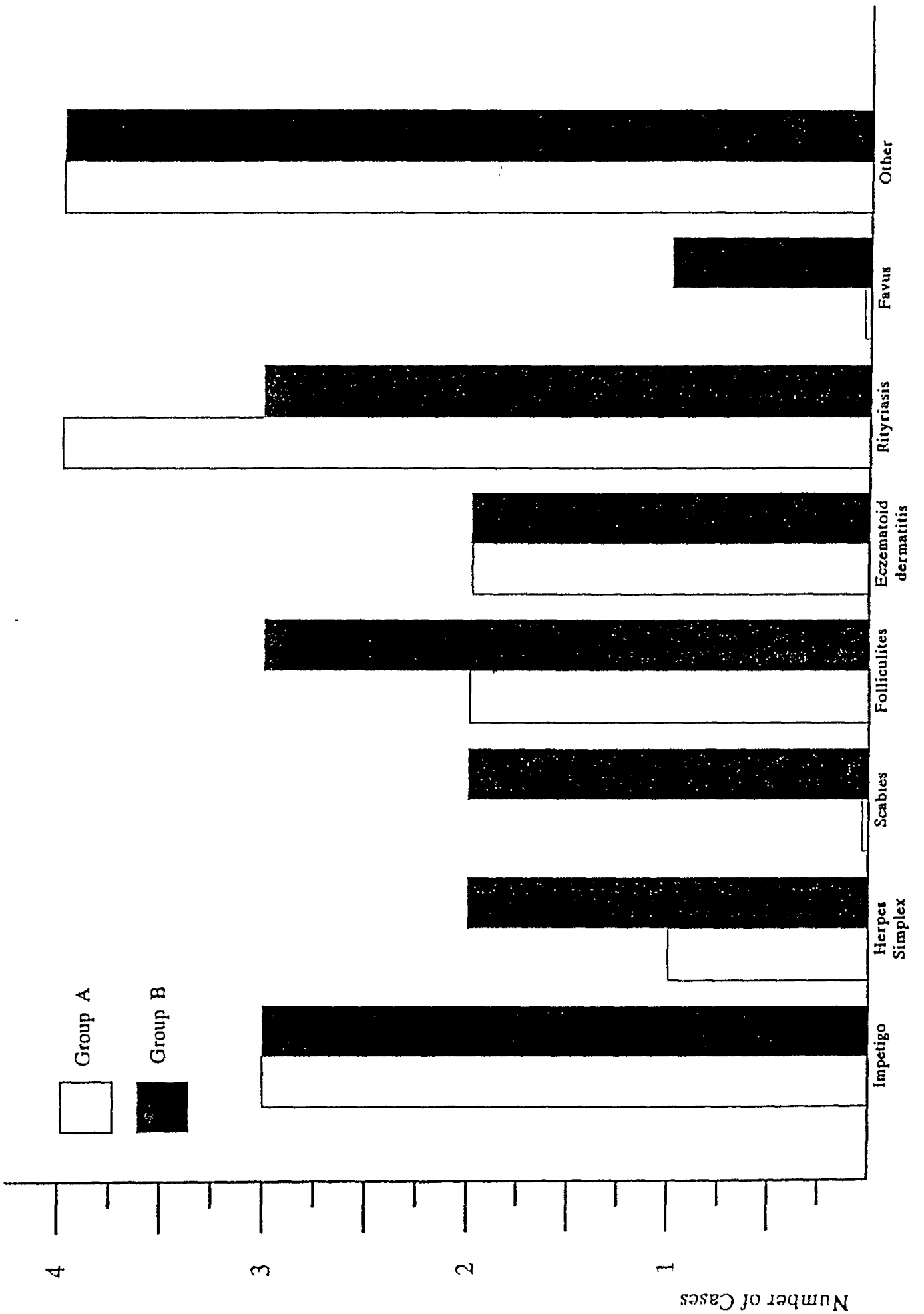

Figure 4. Distribution of dermal diseases in two groups of Thira

4.3.2 Of the 200 cases developed during the summer of 1987 and 1988, 94(47%) at least had taken one bath in the sea before the development of the problem while 106(53%) had taken no bath for at least for 10 days before the dermal problem.

Table 5

Relation between dermal diseases and swimming from Dermatological Hospital

Total cases in years 1987-88	One or more bath	No bath
200	94(47%)	106(53%)

It can be seen that one or more baths did not increase the incidence of dermal disease (table 5).

5. DISCUSSION

Previous studies showed that there is a relation between bacterial contamination of the seawater and diseases in man mainly of the gastrointestinal tract (Seyfried *et al.* 1985a, Fattal and Shuval 1988). There was however no proven positive relation between contamination and dermal diseases (Seyfried *et al.* 1985b), Fattal and Shuval 1988).

In the study of Fattal and Shuval (1988) it was found that the incidence rate of respiratory symptoms was higher among swimmers than among non-swimmers, but did not correlate with bacterial indicator density. This indicates that the morbidity due to respiratory symptoms was not influenced by bacterial seawater pollution, but by the act of swimming. However, a higher incidence rate of dermal disease was not found.

Our study was performed in order to check the possible relation between diseases and sea and sand factors. The frequency of cases in swimmers showed no significant difference with that of non-swimmers. It is characteristic that even at group of swimmers in contaminated water there was no observed increase of cases compared to non-swimmers. This fact is also reported by other investigators (Fattal and Shuval 1988, Seyfried *et al.* 1985a, Seyfried *et al.* 1985b). It has been noted that even in the cases of dermal problems in swimmers, there seems to be no influence from factors related to the sea (sand, sun exposure).

On the other hand, the evaluation of case frequency in two General Hospitals showed no increase during summer. At least, no relation between dermal problems and the sea was noticed in the cases of the special Hospital.

It is obvious that the sea is not responsible for the increase in dermal cases during summer.

The increase in some kinds of dermal problems during summer is apparently related to factors other than the sea, such as sun, etc.

In our study there seems to be no difference in dermal problems caused by bacteria (impetigo), something that would have direct relation to sea contamination. Fungus infection seems to be related more with sand than with the sea. Something that cannot be excluded is the possible action of chemical factors (petroleum components, oils etc.) to sensitization of the skin resulting in long-term disorders. It has to be noted also that in the study by Fattal *et al.* (1988) the highest frequency of intestinal infection was mainly in the age group of 0 to 9 years. For this age group, it is suggested that other factors, such as epidemiology of intestinal infections and immunological conditions, are considered to be basic for the development of these cases.

In our study there were no individuals of this age group and therefore no control of morbidity was done.

6. CONCLUSIONS

- The basic scope of this study was to find out the possible influence of sea bathing to the development of dermal diseases.

- The percentage of cases during the summer months does not differ significantly between the groups of swimmers and non-swimmers as well as swimmers in contaminated water in the Attica area.

- The same difference was noted in Thira island between the two groups of swimmers and non-swimmers where there is no contamination.

- There is no increase of dermal cases during the summer months as compared to those of winter, according to the data of two General Hospitals.

- In 200 cases during summer from special Dermatological Hospitals, it was reported that 43% had sea contact by swimming and 57% had not.

In general, the sea seems to cause no serious problems to the skin even in places that are contaminated (high % of faecal coliform). However, we must not exclude the action of factors that are related to the sea, such as sand, exposure to sun etc., to the development of dermal problems. This has to be examined with special study protocols.

During summer there is an increased rate of dermal problems due to known reasons such as insect bites, action of prolonged exposure to sun, etc.

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APPENDIX I

QUESTIONNAIRE A

Information for dermal diseases in swimmers and non swimmers in Attica.

A.

1. Sex: Male ~ Female ~

2. Age (years): 16-25 ~ 25-35 ~ 36-45 ~ > 46 ~

3. District of Attica: Athens ~ Piraues ~ Suburbs ~

B.

1. Did you swim in the sea during the period of June-September 1987:

   Yes ~ No ~

2. If No:

   a. Did you suffer from any skin disease during the same period:

      Yes ~ No ~

   b. If yes, what was it:

      Impetigo
      Herpes Simplex
      Scabies
      Folliculites
      Eczematoid dermatits
      Pityriasis
      Favus
      Other

   c. In which month: June ~ July ~ August ~ Sept. ~

   d. Has a doctor seen you: Yes ~ No ~

   e. Did you have any exposure to sun apart from the usual:

      Yes ~ No ~

3. If you were a swimmer:

   a. How many times you swim during the above period

      < 10 ~ >10 ~

   b. If you swam ten or more times:

   c. Did you suffer from any skin disease:

      Yes ~ No ~

   d. If yes, what was it:

      Impetigo
      Herpes Simplex
      Scabies
      Folliculites
      Eczematoid dermatitis
      Pityriasis
      Favus
      Other

   e. The skin disease has appeared after swimming:

      2 times ~ 5 times ~ 10 ~ or > 10 ~

   f. Has a doctor seen you: Yes ~ No ~

   g. Did you have a shower after swimming: Yes ~ No ~

4. Where did you swim (indicate the correct area if possible)
   (The last question was used to identify the pollutant areas).

APPENDIX II

QUESTIONNAIRE B

Information for dermal diseases in swimmers and non swimmers in island of Thira.

A.

1. Sex: Male ~ Female ~

2. Age (years): 16-25 ~ 25-35 ~ 36-45 ~ > 46 ~

3. District of Attica: Fira ~ Ia ~ Other ~

B.

1. Did you swim in the sea during the period of June-September 1988:

   Yes ~ No ~

2. If No:

   a. Did you suffer from any skin disease during the same period:

      Yes ~ No ~

   b. If yes, what was it:

      Impetigo
      Herpes Simplex
      Scabies
      Folliculites
      Eczematoid dermatits
      Pityriasis
      Favus
      Other

   c. In which month: June ~ July ~ August ~ Sept. ~

   d. Has a doctor seen you: Yes ~ No ~

   e. Did you have any exposure to sun apart from the usual:

      Yes ~ No ~

3. If you were a swimmer:

a. How many times you swim during the above period

< 10 ~ >10 ~

b. If you swam ten or more times:

c. Did you suffer from any skin disease:

Yes ~ No ~

d. If yes, what was it:

Impetigo
Herpes Simplex
Scabies
Folliculites
Eczematoid dermatitis
Pityriasis
Favus
Other

e. The skin disease has appeared after swimming:

2 times ~ 5 times ~ 10 ~ or > 10 ~

f. Has a doctor seen you: Yes ~ No ~

g. Did you have a shower after swimming: Yes ~ No ~

i. Did you lie on the sand after the swimming: Yes ~ No ~

DIETARY METHYLMERCURY INTAKE AND THE HUMAN EXPOSURE IN AN ADRIATIC POPULATION

by

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1. INTRODUCTION

The assessment of the state of pollution of the Mediterranean Sea by mercury indicated that certain populations in the Mediterranean region could have an intake of methylmercury in excess of tolerable levels through seafood (UNEP, 1987). A project on evaluation of methylmercury in Mediterranean populations and related health hazards was therefore developed by WHO in collaboration with FAO and UNEP. The main components of this project consisted in so-called "sensitive areas" and the examination of dietary mercury intake as well as the hair mercury content in population samples.

In 1989 we reported that the dietary mercury content in an area of the Adriatic Sea polluted with inorganic mercury from a local industry plant, was much higher than in an area without industry. This difference was particularly pronounced with regard to total mercury, but the difference in methylmercury was much smaller and did not reach the level of statistical significance (Buzina *et al.*, 1989). Consequently, the percentage of subjects ingesting total mercury from seafood above the FAO/WHO Provisional Tolerable Weekly Intake (PTWI) of 300 microgrammes was also higher in the industrially polluted area. On the other hand, the percentage of subjects whose methylmercury intake was above the PTWI of 200 microgrammes for methylmercury, was higher in the control area mainly due to the increased number of subjects consuming seafood more than 5 times a week. In addition, the question was also raised whether the control region, although industry-free, was really an entirely unpolluted area as it was no more than 3 km away from the mainland.

We decided, therefore, to continue to study the mercury content of seafood as well as dietary mercury intake in the apparently non-polluted areas by selecting one of the most distant islands off the Adriatic coast of Croatia with no local industry except for a fish processing plant.

2. SELECTION OF POPULATION

The study was carried out on the island of Vis located about 80 km from mainland Croatia. Since the island was closed to international tourism for many years, which affected the economic development, there was a significant exodus of particularly younger people who went to the mainland in search of jobs and/or higher education. Therefore, a substantial proportion of the population were older, and retired persons. Some of the residents were employed in the local fish processing plant or in administration and there were also a few self-employed craftsman. Professional fishing was well developed but most of the residents have also taken up fishing for personal and family consumption.

The selection of households for the study was based on the town of Vis roster, with those engaged in fishing professionally or semi-professionally being selected first. Consequently, the sample was not a random one, but was drawn primarily from those groups known to be more exposed to mercury so as to identify the population sector requiring most attention. The same criterion has also been applied to the selection of populations in the previous study (Buzina *et al.*, 1989).

Thirty three households with 92 family members were included in the study. Thirty percent of selected subjects belonged to the families of professional fishermen. The study was carried out for two weeks in April 1990.

3. METHODS

3.1 Dietary Surveys

Dietary surveys were carried out in two phases. In the first phase, families were interviewed about their seafood consumption habits, including the species of fish and other seafood consumed throughout the year. In the second phase, the seafood consumption pattern of the family, according to the type and quantity of seafood consumed during the two week household survey, was recorded. Specially designed forms prepared by the MED POL Phase II programme were used (WHO, 1982). Details of the dietary survey method are described in the previous report (Buzina *et al.*, 1989).

3.2 Determination of Mercury in Seafood

Fish samples were prepared according to UNEP/FAO/IAEA recommendations (UNEP/FAO/IAEA, 1984). All samples were stored frozen until analyzed.

Total mercury was determined in aliquot of homogenized fish (approx. 0.5 g fresh sample) by gold amalgamation cold vapour atomic absorption spectrophotometry (CV AAS) after acid digestion under pressure in closed Pyrex tubes according to the method by Horvat *et al*., 1986. Certified reference materials (CRMs) were analyzed in parallel to actual samples in order to control the quality of data. About 10 per cent of samples were also run in three independent determinations and re-analyzed by neutron activation analysis (Kosta *et al.*, 1969).

Methylmercury was determined by HCL leaching and ion-exchange separation of organic and inorganic mercury followed by gold amalgamation CV AAS (May *et al.*, 1987). CRMs were analyzed together with actual samples. Additionally, the quality of the data was checked by comparing results obtained by gas liquid chromatography (Horvat *et al.*, 1988, 1990).

3.3 Determination of Mercury in Hair

Hair samples were cut as close as possible to the scalp in the occipital area in an amount of approximately 0.5 g. The hair was collected on a sheet of white paper with proximal ends on the same side, and subsequently transferred into a paper bag for shipping to the laboratory. Hair samples were then cut into 2-5 mm segments and cleaned according to the IAEA, WHO and UNEP recommendations (IAREA, 1978; UNEP/WHO/IAEA, 1987).

Total mercury and methylmercury were determined by gold amalgamation CV AAS as described for fish samples.

The analyses of seafood and hair mercury were carried out by the Department of Nuclear Chemistry of the "Josef Stefan" Institute, Ljubljana, Slovenia.

4. STATISTICAL ANALYSIS

Descriptive statistics were used to calculate the age and sex-specific medians, means and standard deviations. Inverse regression (Draper *et al.*, 1981) was used to estimate the weekly seafood consumption corresponding to the PTWIs for total and methylmercury. Because of the skewness of the distributions of seafood consumption of total and methylmercury intake, the square root transformation was used before the regression lines were computed. This resulted in more symmetric and approximately normal distribution of the residuals from the fitted regression lines. The estimated means and confidence intervals were then transformed back to the original scale. All analyses were performed by using the Statistical Package for Social Sciences (SPSS/PC+ SPSS, Inc. Chicago).

5. RESULTS

A total of 92 subjects were examined (47 male and 45 female). Their ages ranged from 2 to 83 years, with 13 (14%) under the age of 16 years. The average weekly consumption of seafood ranged from 1 to 7 times per week with a mean seafood consumption of 4.5 times per week. Adults consumed seafood more frequently than children and males tended to consume seafood more frequently than females (Table 1 and Figure 1). Altogether 37 subjects (40%) consumed seafood 6 or more times per week. The most commonly consumed fish were Spanish Mackerel (*Scomber Japonicus*), Pilchard (*Sardina Pilchardus*) and bogue (*Boops Boops*). Table 2 shows for those seafood consumed by more than 10% of families the total and methylmercury content of samples taken from the Vis area and compares the total and methylmercury content of the same seafood taken from the previously reported industrially polluted area of Kastela Bay. The total mercury seafood content was slightly lower in the Vis area, but methylmercury content was in most cases slightly higher. This is in agreement with the results of our earlier study showing that industrial pollution affected primarily the total mercury seafood content and, to a lesser extent, the methylmercury content.

The mean weekly total intake of seafood was 991 g and was higher in males than females (Table 3). Children under the age of 6 had much lower consumption. The individual consumption is shown in Figure 2 according to sex and age group. The estimated total and methylmercury intakes (Table 3) showed similar patterns, with males having higher intake than females, children under 6 having the lowest intake, and no trend of increasing intake with age. A total of 20 subjects (22%) consumed above the PTWI of 300 µg total mercury and the same number above the PTWI of 200 µg methylmercury (Figure 3(a) and 3(b)). In order to estimate the total seafood intake corresponding to the PTWI levels for total and methylmercury, the regressions of total and methylmercury on the weekly seafood consumption were fitted (Figures 4(a) and 4(b)). The estimated seafood consumption corresponding to a mean intake of 300 µg was 1559 g (95% confidence interval 1,422-1,734 g) and 1,365 g (95% confidence interval 1,266 - 1,485 g) for 200 µg methylmercury.

There was an increased tendency for mean total and methylmercury to increase with increasing seafood consumption and mercury intake, but neither the differences nor the trends were statistically significant. Also, subjects with weekly total and methylmercury intakes above the PTWI did not have significantly higher levels of hair mercury (Table 4).

6. DISCUSSION

While, in the industrially polluted area, the seafood total mercury content was slightly but consistently higher than in the non-industrial areas of the Adriatic, the differences in methylmercury content were less consistent. Seafood in the Vis area contained, in many instances, even higher methylmercury content than in the industrially polluted area. By and large, however, the mercury content of the seafood in the industrially polluted area of Kastela Bay, as well as in the industrially non-polluted areas was well within the range found in other Adriatic regions (UNEP, 1987). Thus the mercury, and notably the methylmercury content of seafood in the examined areas, reflects primarily the general ecological characteristics of the Adriatic, rather than the impact of specific local pollution.

Seafood is an important component of the diet of the Vis population, and it is the major source of animal protein. It is also the cheapest source for those who are engaged in fishing. More than half of the surveyed households were consuming fish more than four times weekly. As a result, however, 20% of the subjects had a mercury intake above the PTWI for both total mercury as well as methylmercury. On the basis of the dietary mercury intake, they should be considered at risk regarding potential health effects (WHO, 1972).

However, the real health significance of the increased dietary mercury intake in subjects exceeding the PTWI cannot be easily assessed. The Provisional Tolerable Weekly Intake as defined by JECFA (WHO, 1987) represents the maximum acceptable level of a contaminant in the diet having the ability to accumulate within the body over a period of time, so that prolonged exposure to the contaminant could be prevented. Therefore, effects are only to be expected if an intake above the PTWI is exceeded for periods of ingestion lasting over months or years.

The presence of methylmercury poisoning can only be diagnosed clinically. It is well established that the nervous system is the principal target tissue in humans. The earliest effects, such as complaints of paraesthesia, malaise and blurred vision, are however non-specific. In man, methylmercury levels in blood cells and in hair provide the best indices of exposure of the nervous system to methylmercury compounds (Draper *et al.*, 1981). Blood levels reflect more accurately the intake from recent exposure to methylmercury, while hair levels reflect the average intake over a more prolonged period. The estimation of mercury in hair may therefore be of value in epidemiological investigation.

Mercury poisoning in the Niigate area in Japan occurred at a mercury level in hair of between 200 to 100 µg/g but, in one case, the hair level was as low as 50 µg/g . In the Iraqi outbreak, levels between 50 and 200 µg/g of methylmercury were found to give rise to risks for symptoms of paraesthesia and were associated with the methylmercury long-term daily intake of 3-7 µg per kg of body weight or 1,260-2,940 µg weekly (Nordberg *et al.*, 1982). However, none of the individuals on the island of Vis, consuming mercury above the PTWI, had hair mercury contents over the critical level at which toxic damage could be expected. Subjects with methylmercury intake above PTWI had maximal (maximum) weekly dietary methylmercury

intake of 833 µg or 4.2 times higher than the PTWI, and had methylmercury levels in hair between 1.1-10.8 µg/g (mean=5.4).

As seafood consumption depends not only primarily on the availability of foods, but also on the dietary habits which are acquired usually in early life, it could be speculated that most of the older subjects with higher fish consumption have been exposed to elevated amounts of mercury for most of their adult life. Yet hair mercury content in subjects with methylmercury intake above the PTWI did not increase significantly after the age of 35. The mean value of hair methylmercury at the age of 35 was 4.1 µg/g (range: 1.1-9.5 µg/g), and 5.4 µg/g (range: 1.2-9.8) after the age of 56. These data show that there was no further substantial accumulation of mercury in the body with increasing age, and the hair mercury content did not approach the levels found in the populations with health impairment.

We have not carried out the clinical examination of the subjects. However, as practically the entire population of Vis is covered by health insurance, it could be expected that some of the early signs of mercury toxicity would have been recorded by local physicians. No such information was, however, reported. Similarly, in a study of 16 subjects from an industrially polluted area in Italy with hair mercury concentration between 4-110 µg, Bacci *et al.* (1987) did not observe symptoms of mercury toxicity.

Based on seafood mercury content, the results of this study confirm that the elevated mercury content in the Adriatic Sea is likely to be the result of natural sources, and that anthropogenic contribution may be only secondary and of local importance.

It can also be concluded that, for the time being in the Adriatic regions of Croatia, despite increased mercury intake above the PTWI in segments of populations studied, the neuropsychological changes in adult populations, if any, were likely to be insignificant. The obtained data also do not suggest that fish consumption in adult populations should be discouraged even if taken on a daily basis. The unresolved question remains, however, the effect of mercury intake on pregnant women, consequently on newborn babies.

The results of this study also support the proposal that hair mercury level of 25 ppm should be adopted for identification of individuals possibly at risk (WHO/FAO/UNEP, 1986).

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Table 1

Weekly seafood consumption by age and sex
(average number of times seafood is consumed per week)

Age group (years)	Males			Females			All subjects		
	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD
1-5	2	3.5	.7	2	2.5	.7	4	3.0	.8
6-14	4	4.8	2.1	5	4.6	1.7	9	4.7	1.8
15-29	9	4.0	2.5	4	2.8	1.3	13	3.6	2.3
30-44	10	3.9	2.4	8	3.6	2.5	18	3.8	2.4
45-59	9	5.6	1.8	14	5.5	1.7	23	5.5	1.7
>60	13	4.8	1.7	12	4.3	2.2	25	4.6	1.9
All ages	47	4.6	2.1	45	4.4	2.1	92	4.5	2.2

Table 2

Total and Methylmercury content (μg/Kg^{-1}) of the most commonly consumed seafood in the diet of the examined families and percentage of families (%) consuming each of the listed species during the period of dietary survey compared with the mercury content of the industrially polluted Kastela Bay

English Name	Latin Name	VIS			KASTELA BAY	
		Total	Methyl	%	Total	Methyl
Span. Mackerel	*Scomber Jap.*	66	66	78.8	80	22
Pilchard	*Sardina Pilch.*	180	139	33.3	198	55
Bogue	*Boops Boops*	267	224	30.3	312	122
Mackerel	*Scomber Scomber*	70	16	24.2	81	69
Comm. Octopus	*Octopus Vulg.*	364	320	21.2	520	495
Mullet	*Mullus SPP*	370	350	18.2	318	128
Scorpion Fish	*Scorp. Scrofa*	222	220	15.2	390	170
Saddled Bream	*Oblada Melan.*	145	129	15.2	174	96
Common Squid	*Loligo Vulg.*	278	249	15.2	322	194
Norw. Lobster	*Nephrops Norv.*	480	430	12.1	540	508
Ann.Git Head	*Diplodus Ann.*	653	502	12.1	628	321
Sea Eel	*Conger Conger*	431	407	12.1	152	139
Goldlin	*Salpa Salpa*	9	8	12.1	54	11

Table 3

Weekly intake of intake of seafood(g), total Mercury(µg) and Methylmercury(µg) by age and sex

AGE GROUP (years)	MALES			FEMALES			ALL SUBJECTS		
	N	MEAN	STD	N	MEAN	STD	N	MEAN	STD
Fish intake(g)									
1-5	2	153	120	2	173	115	4	163	97
6-14	4	1246	725	5	956	438	9	1085	563
15-29	9	1021	606	4	628	291	13	900	549
30-44	10	1325	928	8	742	605	18	1066	834
45-59	9	1393	687	14	885	394	23	1084	573
>60	13	1191	526	12	787	421	25	997	512
All ages	47	1186	697	45	787	445	92	991	618
Total Mercury (µg)									
1-5	2	28	32	2	15	10	4	22	21
6-14	4	277	226	5	230	162	9	251	182
15-29	9	184	133	4	132	17	13	168	111
30-44	10	294	234	8	170	157	18	239	208
45-59	9	282	263	14	167	157	23	212	207
>60	13	195	136	12	135	110	25	166	125
All ages	47	231	195	45	156	136	92	194	172
Methylmercury (µg)									
1-5	2	11	9	2	11	7	4	11	7
6-14	4	212	170	5	165	113	9	186	133
15-29	9	145	113	4	107	8	13	133	94
30-44	10	186	167	8	131	116	18	161	145
45-59	9	230	241	14	130	144	23	169	189
>60	13	148	124	12	106	102	25	128	114
All ages	47	171	161	45	120	114	92	146	141

Table 4

Hair mercury levels by weekly level of fish, total mercury and methylmercury intake

	N	MEAN	STD	MIN	MAX.
HAIR TOTAL MERCURY					
Fish intake (g/week					
0-999	51	4.91	3.15	.33	16.30
1000-1500	23	6.56	4.67	.84	19.30
>1500	17	6.39	3.51	1.30	12.10
Total Mercury intake (µg/week)					
0-100	27	5.19	3.32	.33	16.30
100-300	44	5.53	3.90	.84	19.30
>300	20	6.32	3.79	1.30	12.90
Methylmercury intake (µg/week)					
0-100	41	5.51	3.39	.33	16.30
100-200	30	5.20	4.10	.84	19.30
>200	20	6.40	3.73	1.30	12.90
HAIR METHYLMERCURY					
Fish intake (g/week)					
0-1000	51	4.15	2.89	.24	14.90
100-1500	23	5.61	4.12	.61	17.10
>1500	17	5.48	2.94	1.13	9.81
Total Mercury intake (µg/week)					
0-100	27	4.46	3.03	.24	14.90
100-300	44	4.71	3.49	.61	17.10
>300	20	5.30	3.27	1.08	10.80
Methylmercury intake (µg/week)					
0-100	41	4.71	3.10	.24	14.90
100-200	30	4.40	3.65	.61	17.10
>200	20	5.43	3.15	1.08	10.80

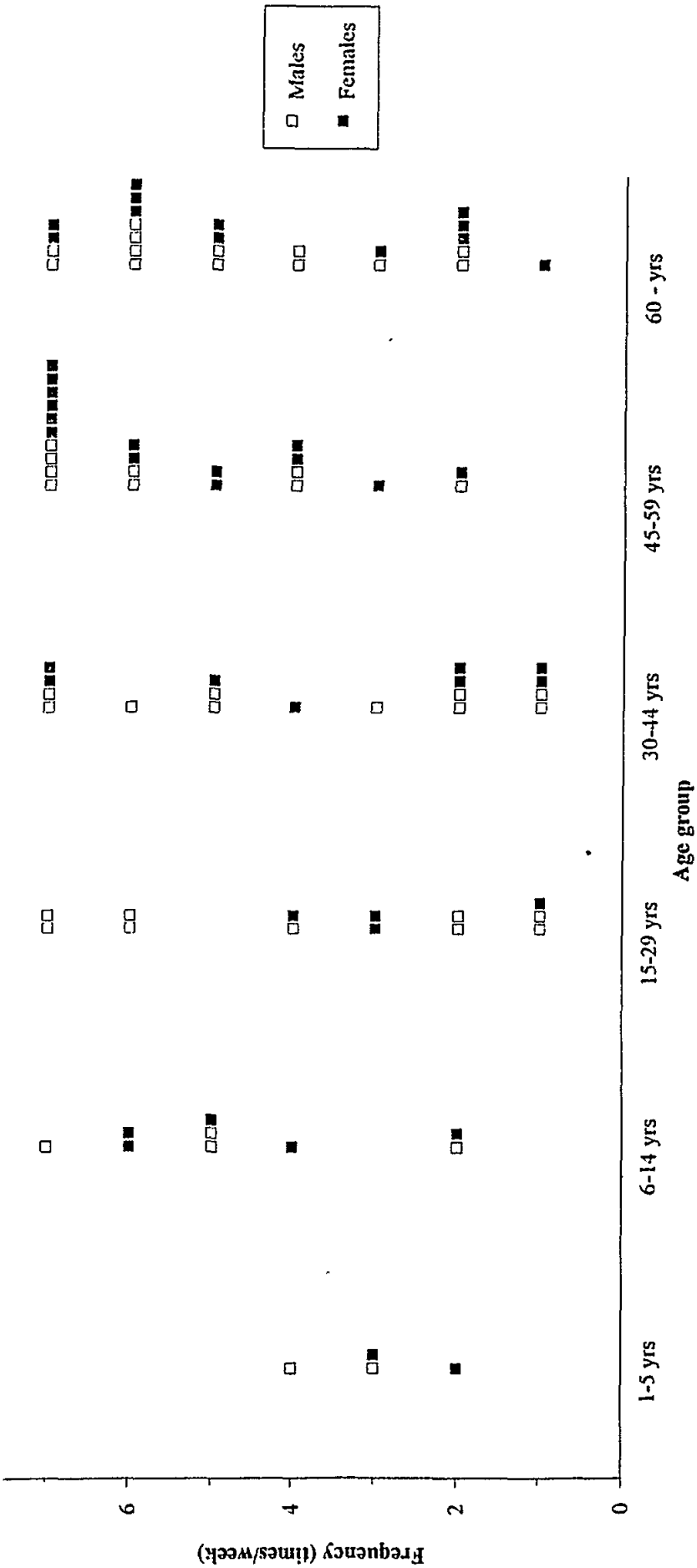

Figure 1. Weekly seafood consumption

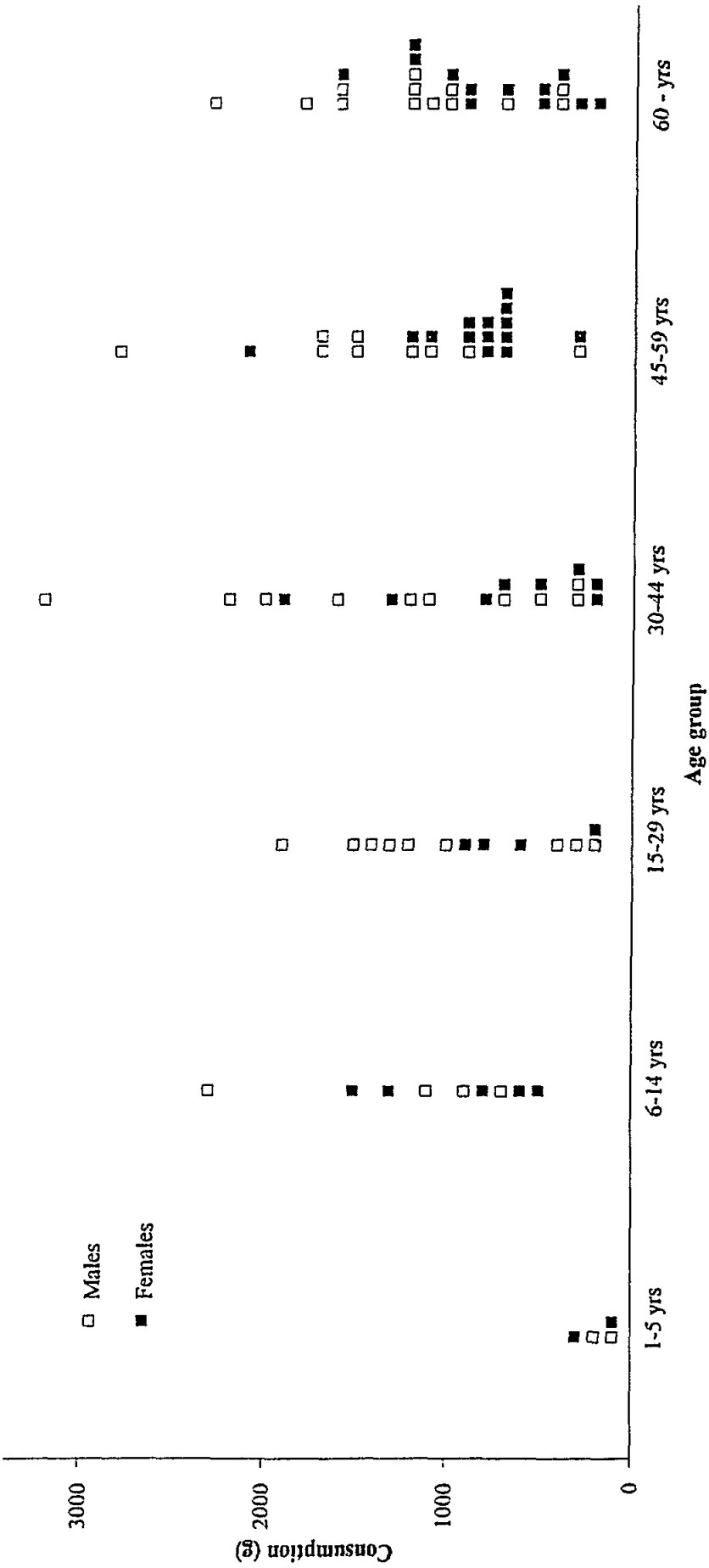

Figure 2. Weekly intake of seafood

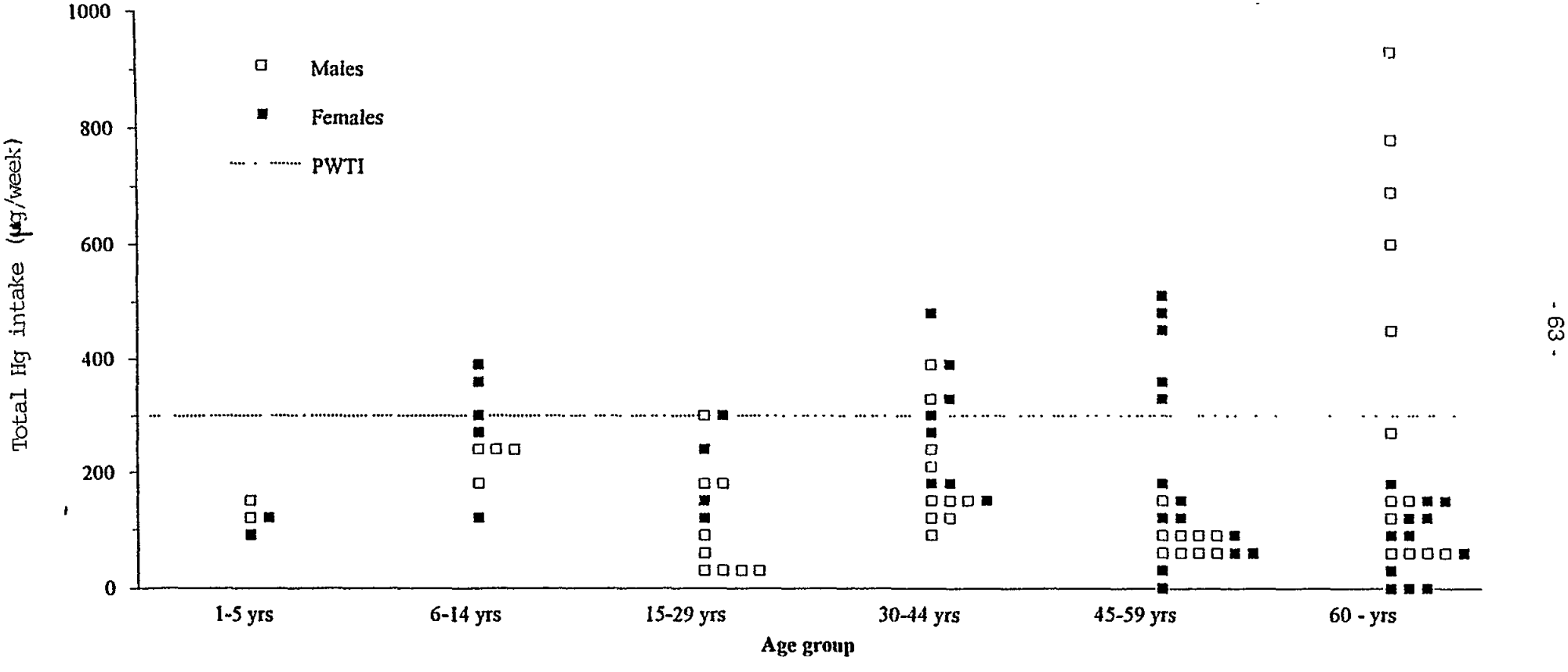

Figure 3a. Weekly intake of Total Hg

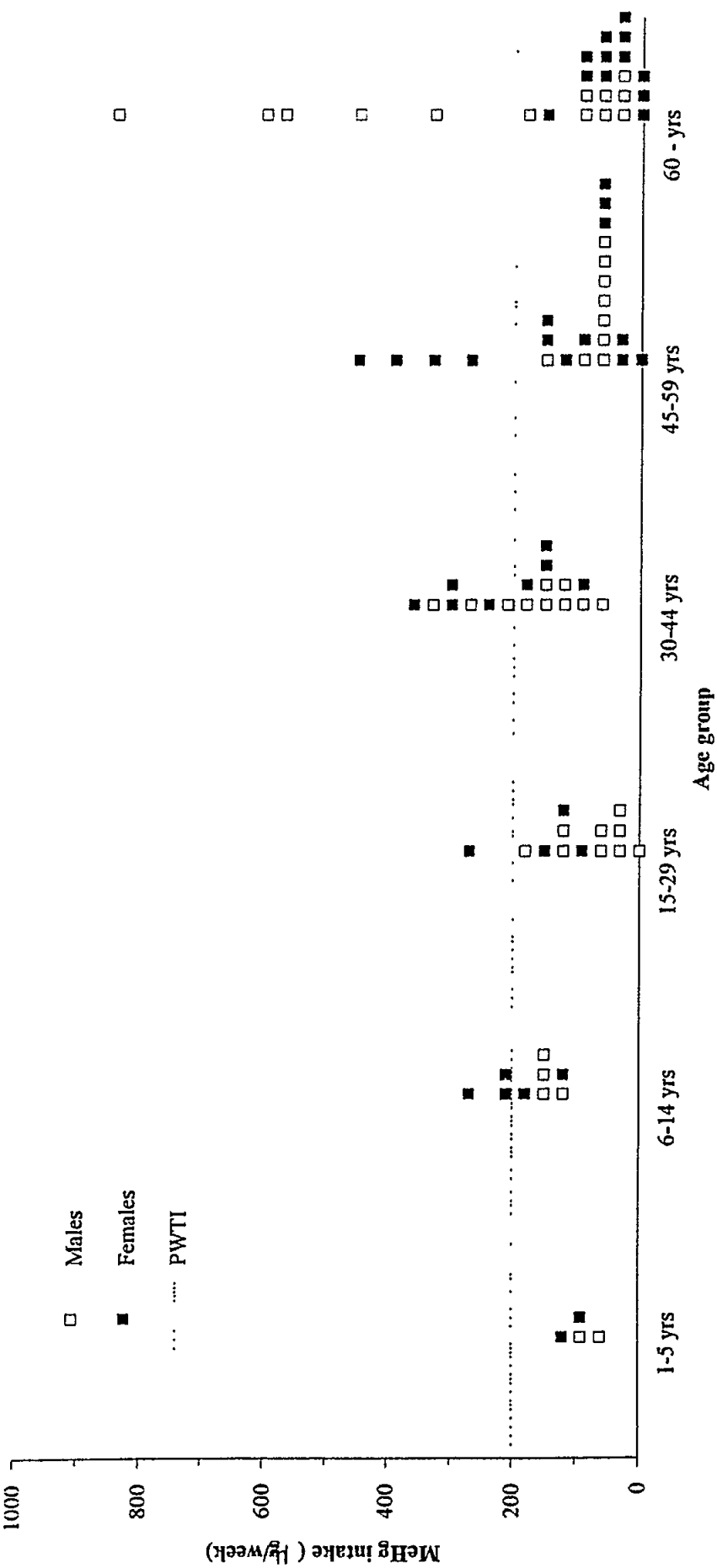

Figure 3b. Weekly intake of methyl Hg

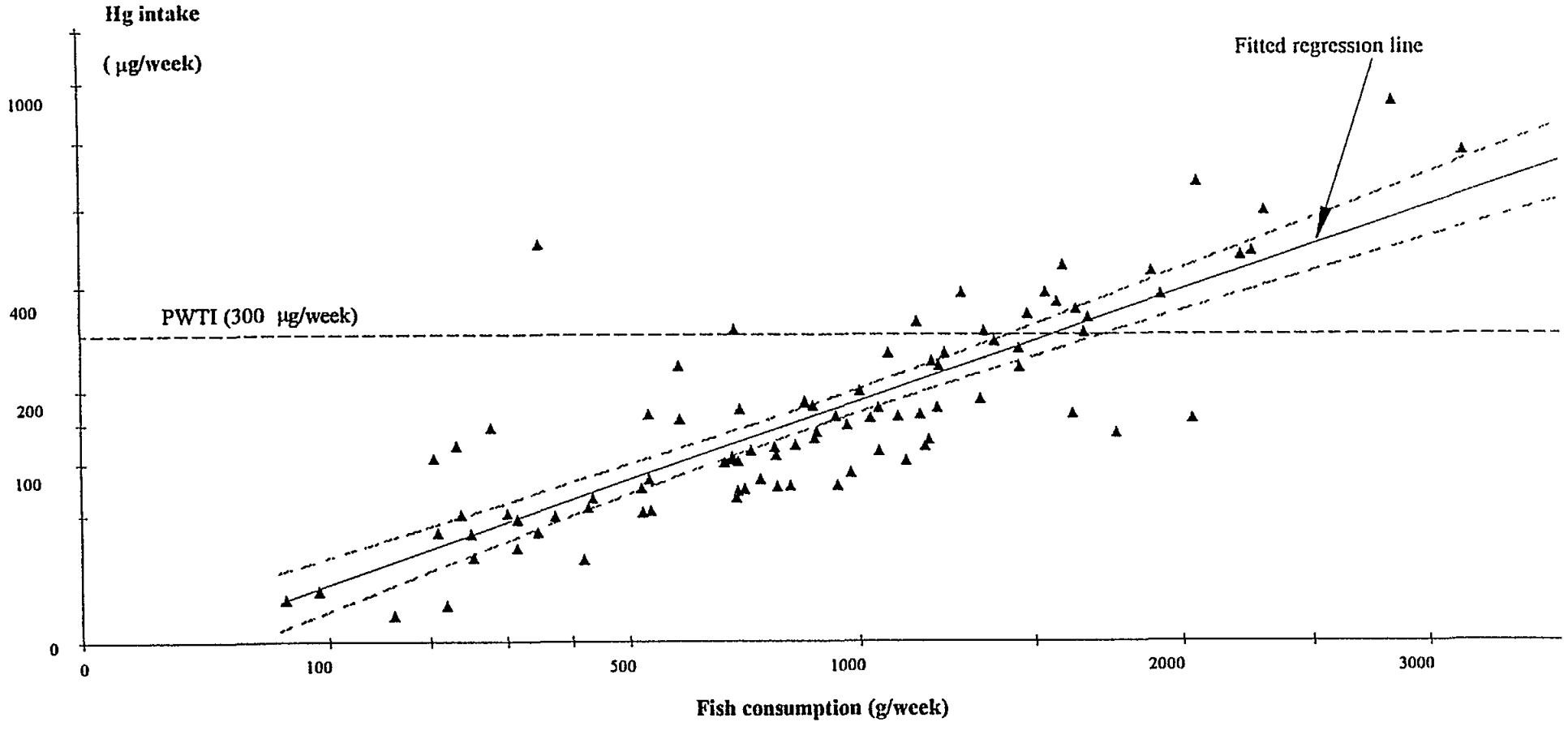

Figure 4a. Total Hg intake and fish consumption (Square root scale)

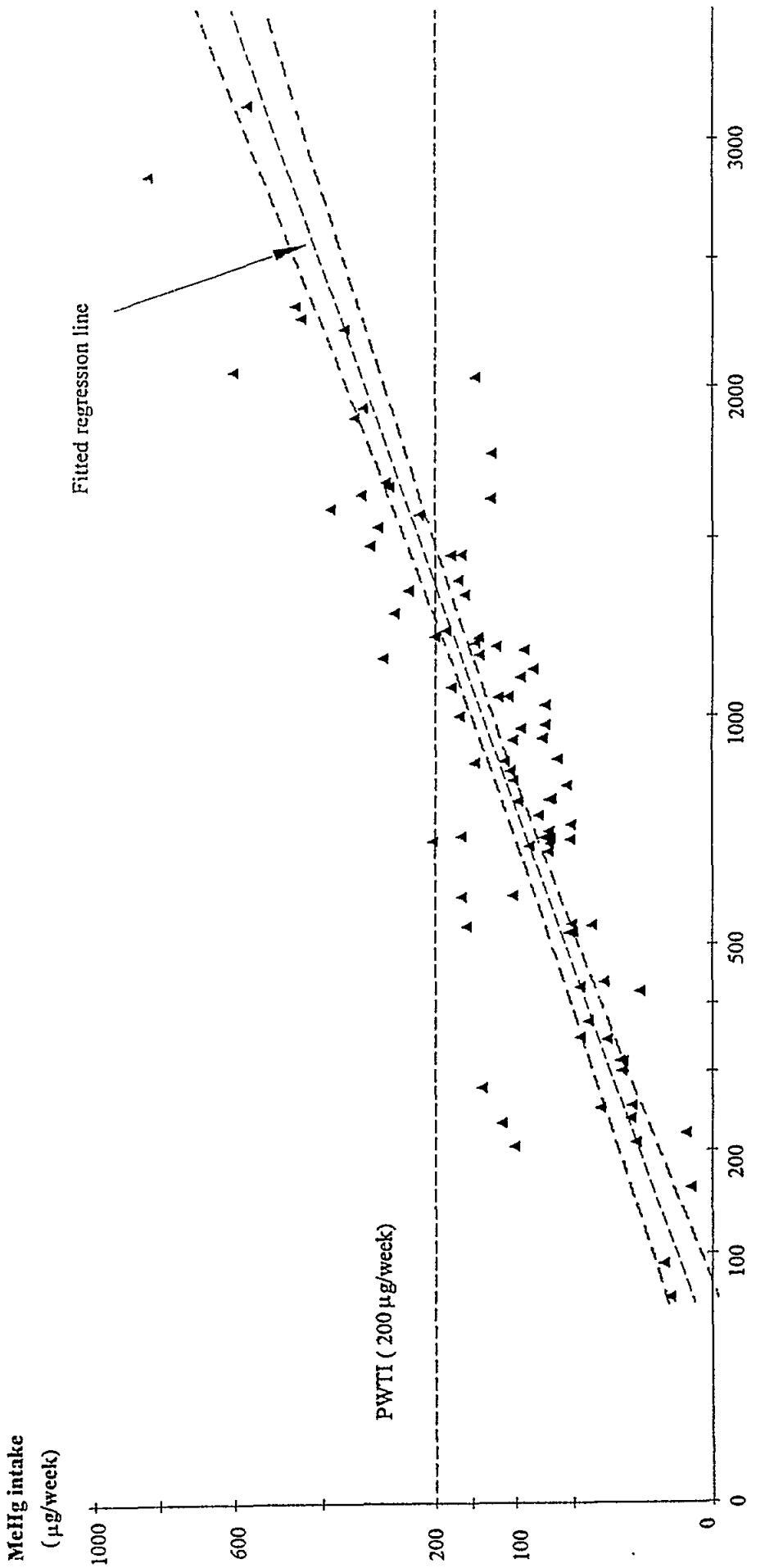

Figure 4b. Methyl Hg intake and fish consumption (Square root scale)

MERCURY LEVELS IN FISHERMAN GROUPS OF THE NORTH ADRIATIC SEA

by

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1. INTRODUCTION

A number of studies of Hg levels in sea organisms, carried out in various areas of the Mediterranean sea up to the early 1970s, indicated that the concentrations in this region are generally higher than those recorded in other seas. The same conclusion was reached with regard to other matrices in the Mediterranean marine environment.

The potential health effects on the population by excessive intake of Hg (particularly methylmercury) have led to considerable international activities (WHO, World Health Organization; FAO, Food and Agricultural Organization; IAEA, International Atomic Energy Agency) in an effort to identify and quantify the problem.

These researches show that most of the general Mediterranean population seem to have a low Hg intake through the consumption of seafood and could be considered not to be at risk (UNEP/FAO, 1987). On the other hand, it appeared equally evident that some population groups in the Mediterranean Region could have an intake of methylmercury through seafood in excess of permissible levels.

The north Adriatic sea receives great amounts of water from rivers that have run through open country and many industrial areas of northern Italy. Moreover, it should be emphasized that Italy is one of the most important producers of Hg and that in the east of the Alps there is a Hg mine (Idrjia) that may influence the waters of small rivers that flow into the north Adriatic sea.

It is known that selenium can play the role of antagonist in mercury toxicity: seafood is one of the main sources of selenium intake (Magos *et al.*, 1988). An inhibition in the mechanism of mercury chelation by the -SH groups in the cell can be suggested in relationship with the antioxidant properties of selenium (Magos *et al.*, 1988).

Besides, as selenium is both essential and toxic, there is an optimum intake to prevent both seleniosis and selenium deficiency: no correlation is demonstrated between high selenium intake and increased risk of cancer, but, on the contrary, it seems that selenium may contribute to prevention of cancer (Alabaster, 1986).

2. MATERIALS AND METHODS

In order to evaluate the possible health risk of the presence of Hg in particular groups of the coastal population of the north Adriatic sea, hair samples of fishermen of different zones were collected to measure the levels of Hg, methylmercury and selenium. On average, fishermen are known to have a greater intake of seafood than other people, but nevertheless, each donor was requested to answer a questionnaire, regarding his/her daily intake of seafood.

Chioggia and Grado (two small coastal towns on the northern side of the Adriatic sea) were selected, for hair sampling, because of their tradition of fishing activity. Hairs were cut with stainless steel scissors, according to the standard method (UNEP/FAO, 1987).

In Chioggia, 77 hair samples were collected from fishermen and their relatives, and, subsequently, another 26 from fishermen only. In Grado, 46 hair samples were collected from individuals of a group of fishermen.

The control population was represented by 92 inhabitants of Sarntal, a mountain district of the Dolomites where food habits do not provide for a significant seafood consumption.

Every month, Hg and methylmercury levels were determined in migratory and non-migratory fish, which represent the most important vehicle for mercury intake. Fish were collected at fish markets and almost all edible species were represented: *Thunnus thynnus, Squalus fernandinus, Mustelus asterias, Lamna nasus, Solea solea, Mullus barbatus, Sparus auratus, Platichtys flesus, Mugil* sp*., Anguilla anguilla, Scomber scombrus*.

The determination of total Hg was carried out by flameless atomic absorption spectrometry (Decr. Min., 1971), whilst the determination of methylmercury was carried out by gas-chromatography, using the partly modified Westoo method (Westoo, 1968).

Selenium analyses were performed using electrothermal atomic spectroscopy with S.T.P.F. conditions, Zeeman background correction and palladium as matrix modifier (Eckerlin *et al.*, 1987).

3. RESULTS

The mean values of the mercury (Hg), methylmercury (MeHg) and selenium (Se) concentrations found in the three considered groups (fishermen and their relatives, fishermen of Chioggia, fishermen of Grado) are represented in Table 1. The standard deviations and the minimum and maximum values are also reported.

Table 2 shows the mean values, standard deviations, minimum and maximum values of Hg and Se of the inhabitants of Sarntal (the control population) and also the Hg and MeHg values of the inhabitants of Chioggia and Caorle (another small coastal town of the north Adriatic sea) that have been investigated in a previous research (Moretti *et al.*, 1990).

Table 3 reports the Hg and MeHg mean values found in 112 non-migratory and in 64 migratory fish of the north Adriatic sea.

In Figures 1, 2 and 3 the Hg and MeHg distributions in the three groups are represented, while Figures 4, 5 and 6 show the correlation between Hg and MeHg in the same groups.

Figure 7 reports the Se distribution in the three groups.

The distribution of Hg and Se in the control population (Sarntal's inhabitants) is represented in Figures 8 and 9, respectively.

4. DISCUSSION

The mean values of the Hg and MeHg levels (Table 1) found in the considered fisherman groups, when compared with those found in other fisherman groups or populations (Kyle *et al.*, 1982; Sherlock *et al.*, 1982), are not considered high, even if some values seem quite elevated.

Each group shows significantly higher Hg and MeHg levels not only in comparison with those of the control population, but also with those of the inhabitants of Chioggia and Caorle (Table 2): it means that fishermen and their relatives are more exposed to Hg than other people.

The Hg and MeHg values found were not homogeneously distributed in the considered population: fishermen who live in the south of the Venetian Region (Chioggia) were shown to have significantly lower levels of Hg than those who live in the north (Grado). In fact, the mean values of these two fisherman groups were, for total Hg, respectively, 3.30 mg/kg and 7.45 mg/kg (Table 1).

If these Hg and MeHg data are compared with those of the inhabitants of Chioggia and Caorle (Table 2), it also appears that the mean values increase gradually from south to north and that fishermen have higher Hg levels than the inhabitants of the same zone.

This could likely depend on the fact that the north of the Region is more influenced by the proximity of the Hg mine of Idrjia, so that the Hg levels of the corresponding waters and of local seafood might be higher, as pointed out by investigations carried out by other Authors (Majori *et al.*, 1976).

On the other hand, the Hg levels shown in groups of fishermen of Chioggia and Grado are significantly lower if compared with those groups in southern Italy (Paccagnella *et al.*, 1973); this difference depends on the fact that in southern Italy fishermen eat large size fish, such as swordfish and scabbard fish, which usually contains higher amounts of Hg than smaller ones.

Meals based on fish were, on average, 3.5 per week and a good relationship was found between the intake of fish and the Hg levels, while no significant difference was observed in different sexes or ages.

The Hg and MeHg levels found in fish in the north Adriatic sea differ according to size and age. The results show that small and young fish have lower Hg and MeHg concentrations than biger older ones, and that food habits play a significant role, with higher Hg concentrations in those fish that feed on crustaceans and other fish.

Non-migratory fish do not show remarkable Hg levels but in several species of migratory fish (as *Thunnus thynnus, Mustelus asterias* and *Lamna nasus*) the mean Hg concentrations exceed abundantly the value of 0.7 mg/kg, which is the limit value permitted by italian law (Table 3).

The three considered groups of fishermen do not differ very much from each other for Hg and MeHg distribution (Figures 1, 2 and 3). In the three groups the greatest percentage of individuals shows values higher than 1 ppm Hg, whilst in the control population (Figure 8) almost all values are included between 0 and 1 ppm Hg. This indicates that Hg is preferentially transmitted by fish or seafood.

More than the 20 percent of the tested fisherman population have a MeHg hair concentration higher than 6 ppm, and the correlation between Hg and MeHg is stronger in the group of fishermen of Grado, with a ratio MeHg/Hg of 84.7%, than in the two groups of Chioggia (Figures 4, 5 and 6).

The Se values, even if in the normal range, seem to be rather low, perhaps because of the presence of Hg: in fact, a correlation with Hg is suspected, as in the group with higher Hg concentrations the Se values are lower, and in the group where Hg is low, Se is higher (Table 1 and Figure 7).

A comparison with the Se levels of the control population (Tables 2 and Figure 9) seems to confirm that there is an inverse proportion between Se and Hg.

In conclusion, even if it seems that, at the moment, there are no great problems for Hg levels for the coastal populations of the north Adriatic sea, biological indicators confirm the critical position of those groups that consume large size fish and those that are situated near the areas at risk.

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Table 1

Mean, standard deviation, minimum and maximum values
of Hg,MeHg and Se levels of some fisherman groups
of the north Adriatic sea

FISHERMEN OF CHIOGGIA AND THEIR RELATIVES (1989, 77 specimens)			
mg/Kg	Hg	MeHg	Se
mean	3.30	1.95	0.70
standard deviation	2.05	1.48	0.27
min. - max values	0.69-10.83	0.28-6.99	0.30-1.86
FISHERMEN OF CHIOGGIA (1990, 26 specimens)			
mg/Kg	Hg	MeHg	Se
mean	3.28	2.29	0.79
standard deviation	1.49	1.44	0.26
min. max values	1.40-7.48	0.46-6.99	0.35-1.28
FISHERMEN OF GRADO (1990-91, 46 specimens)			
mg/Kg	Hg	MeHg	Se
mean	7.45	6.31	0.47
standard deviation	4.54	3.97	0.09
min. 0 max values	1.03-19.87	0.71-18.70	0.10-1.04

Table 2

Mean, standard deviation, minimum and maximum values of Hg, MeHg and Se levels of the inhabitants of Sarntal, Chioggia and Caorle

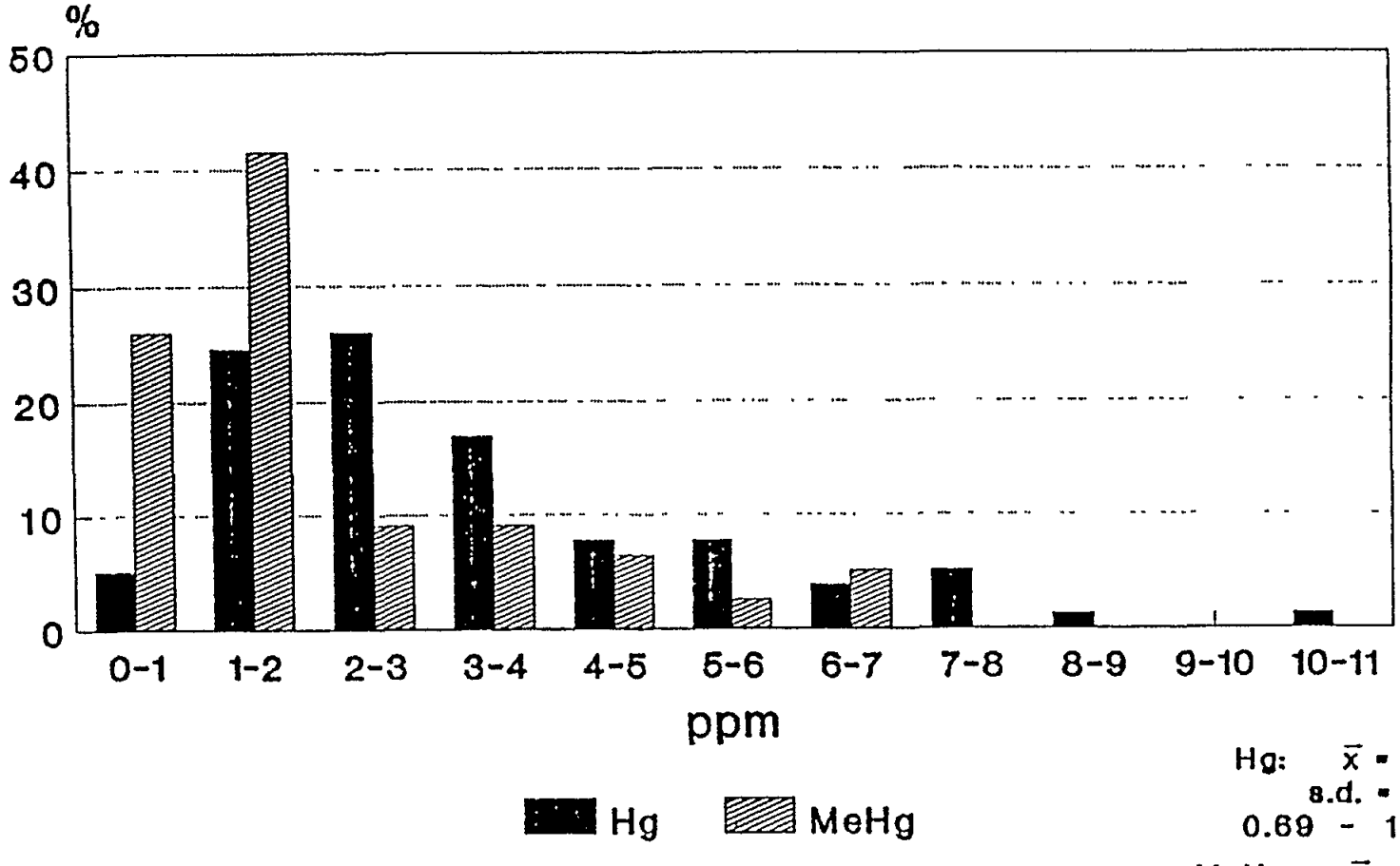
INHABITANTS OF SARNTAL (1989, 92 specimens)		
mg/Kg	Hg	Se
mean	1.40	1.19
standard deviation	1.91	0.48
min. - max values	0.03-12.50	0.50-2.25
INHABITANTS OF CHIOGGIA (1988, 143 specimens)		
mg/kg	Hg	MeHg
mean	2.44	1.45
standard deviation	2.60	1.97
min. - max values	0.10-24.14	0.00-17.28
INHABITANTS OF CAORLE (1989, 296 specimens)		
mg/Kg	Hg	MeHg
mean	3.27	1.36
standard deviation	2.93	1.78
min. - max values	0.20-16.10	0.00-11.82

Table 3

Mean, standard deviation, minimum and maximum values of Hg and MeHg levels of non-migratory and migratory fish of the north Adriatic sea

NON-MIGRATORY FISH (1989-90, 112 specimens)		
mg/Kg	Hg	MeHg
mean	0.34	0.21
standard deviation	0.28	0.22
min. max values	0.01-1.83	0.00-0.88
MIGRATORY FISH (1989-90, 64 specimens)		
mg/Hg	Hg	MeHg
mean	1.32	0.81
standard deviation	0.48	0.34
min. max values	0.33-1.92	0.33-1.22

FISHERMEN AND THEIR RELATIVES
Hg and MeHg Distribution (in %)



Hg: $\bar{x}$ = 3.30
s.d. = 2.05
0.69 - 10.83

MeHg: $\bar{x}$ = 1.95
s.d. = 1.48
0.28 - 6.99

Fishermen of Chioggia, 1989

Figure 1.

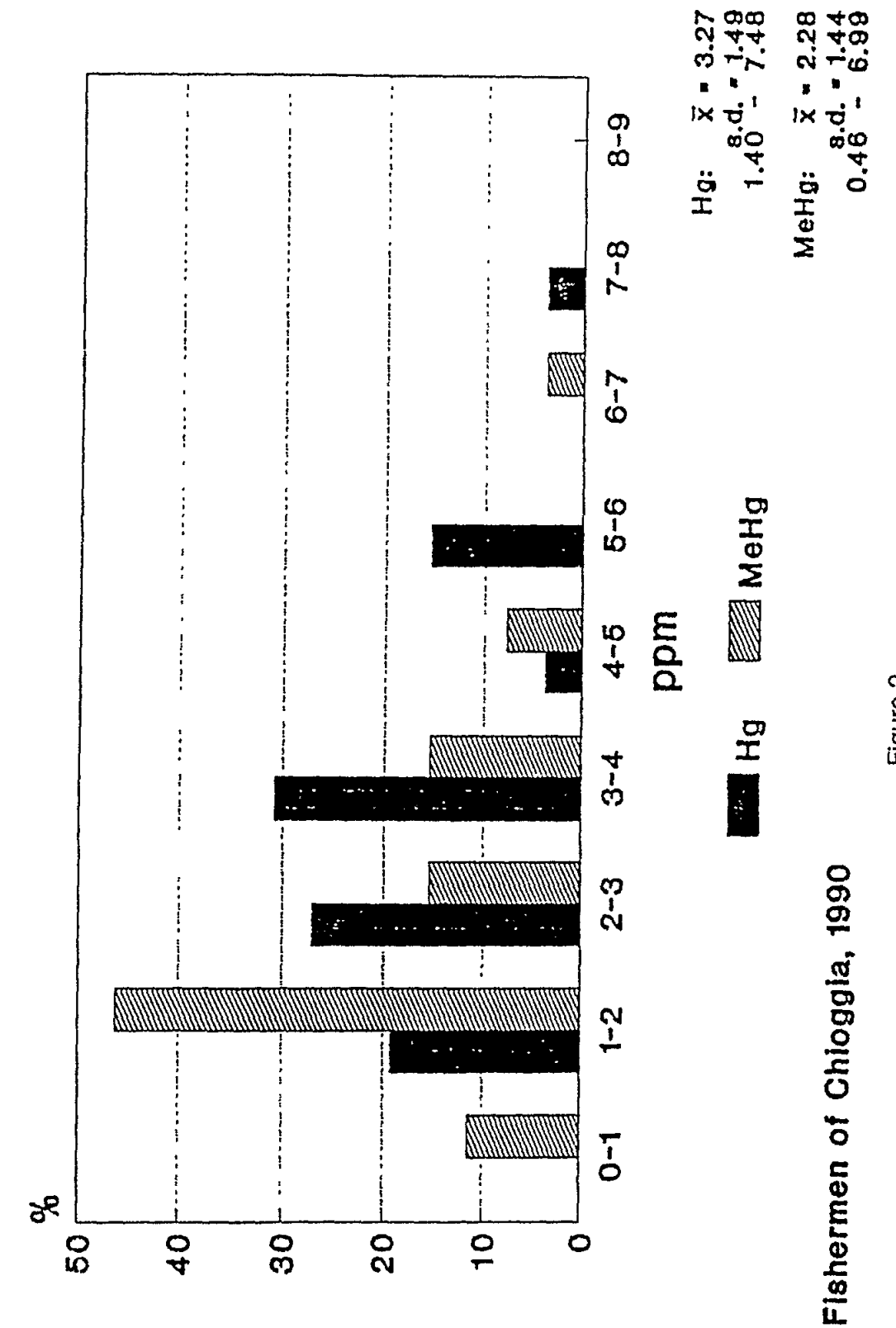
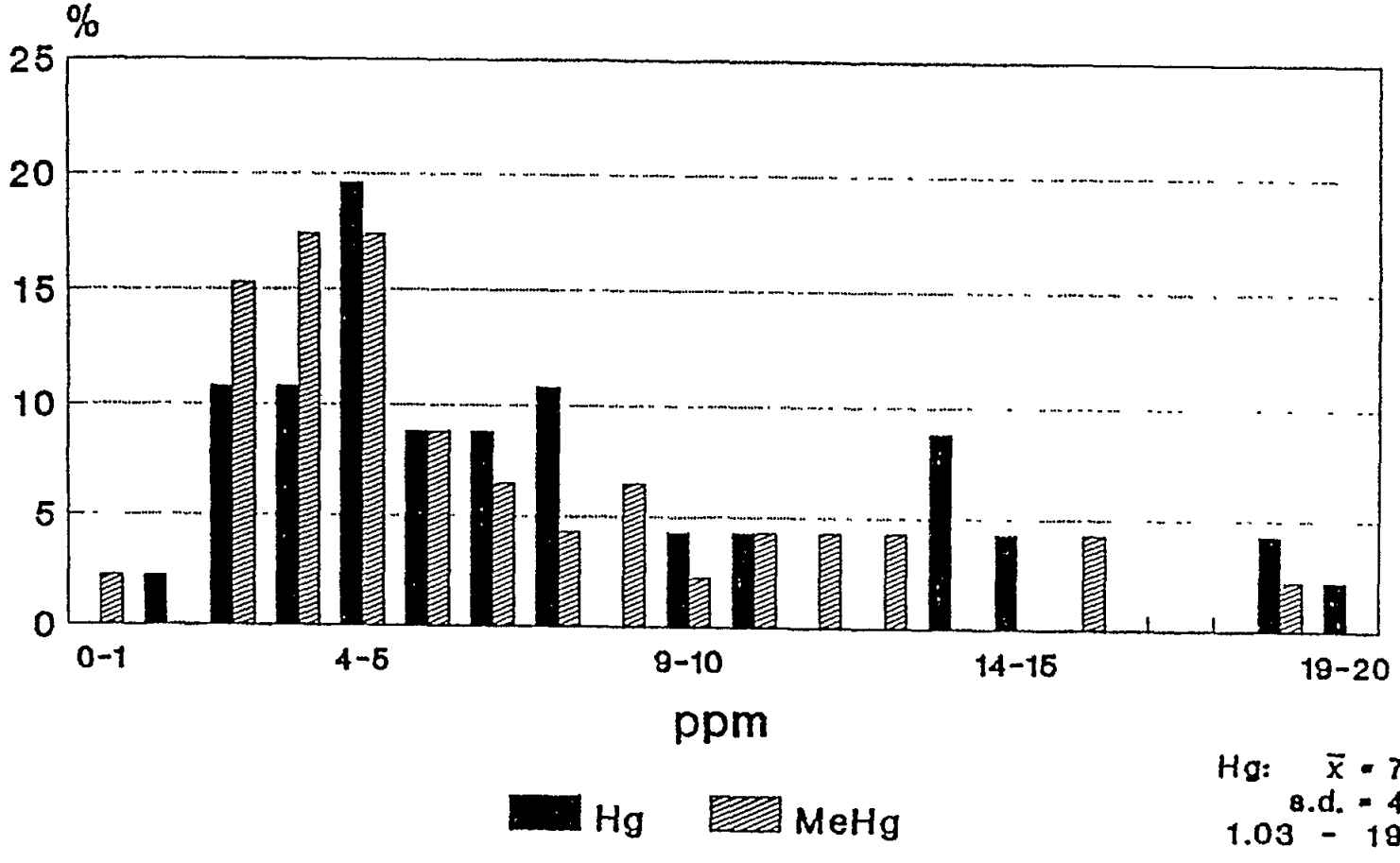

FISHERMEN OF CHIOGGIA
Hg and MeHg Distribution (in %)
%
50
40
30
20
10
0
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
ppm
Hg
MeHg
Hg: x̄ = 3.27
s.d. = 1.49
1.40 - 7.48
MeHg: x̄ = 2.28
s.d. = 1.44
0.46 - 6.99
Fishermen of Chioggia, 1990

Figure 2.

FISHERMEN OF GRADO
Hg and MeHg Distribution (in %)



Hg: $\bar{x}$ = 7.45
s.d. = 4.54
1.03 - 19.87

MeHg: $\bar{x}$ = 6.31
s.d. = 3.97
0.71 - 18.7

Fishermen of Grado, 1990-91

Figure 3.

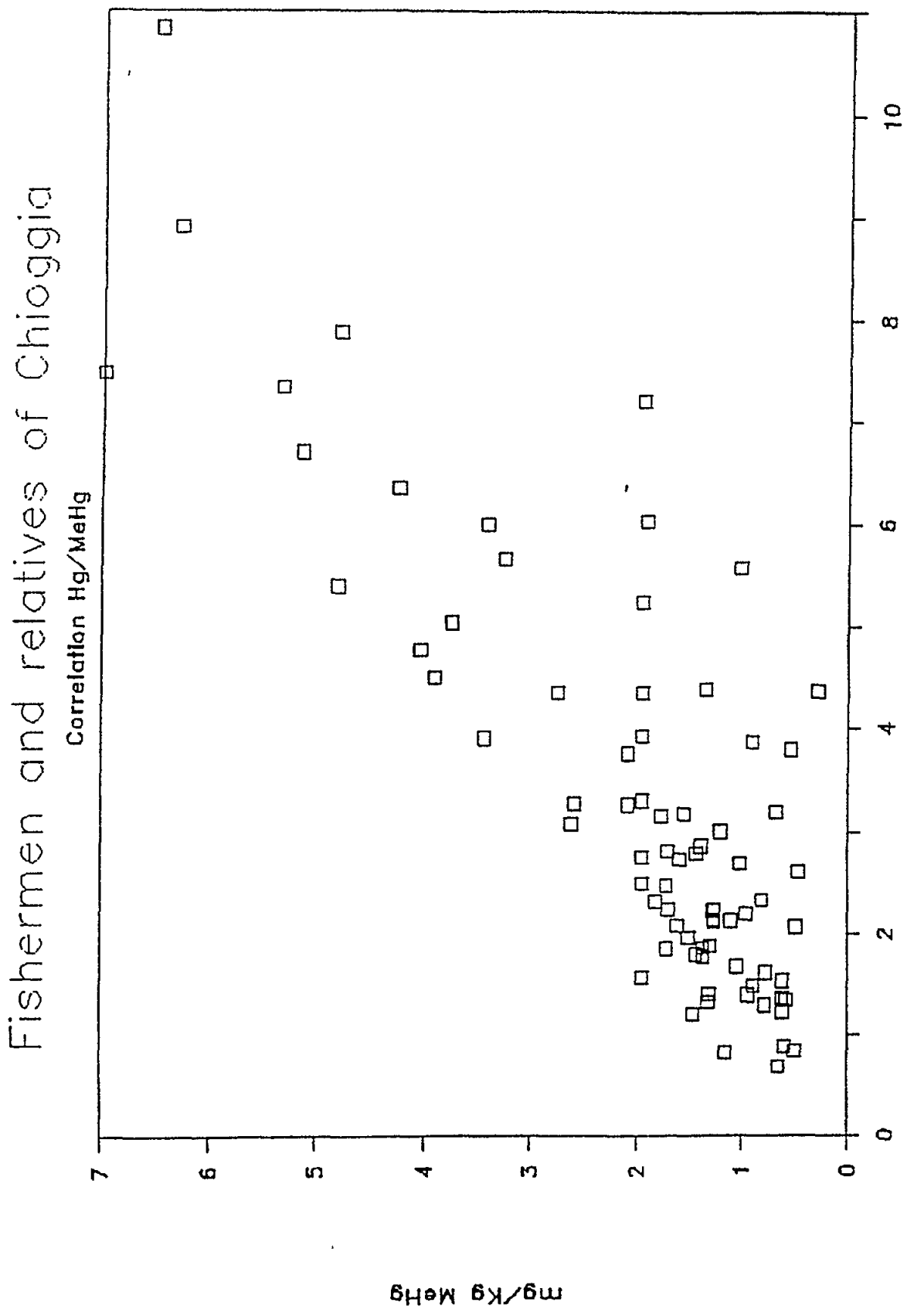

Fishermen and relatives of Chioggia
Correlation Hg/MeHg
mg/Kg MeHg
mg/Kg Hg
0
1
2
3
4
5
6
7
0
2
4
6
8
10

Figure 4.

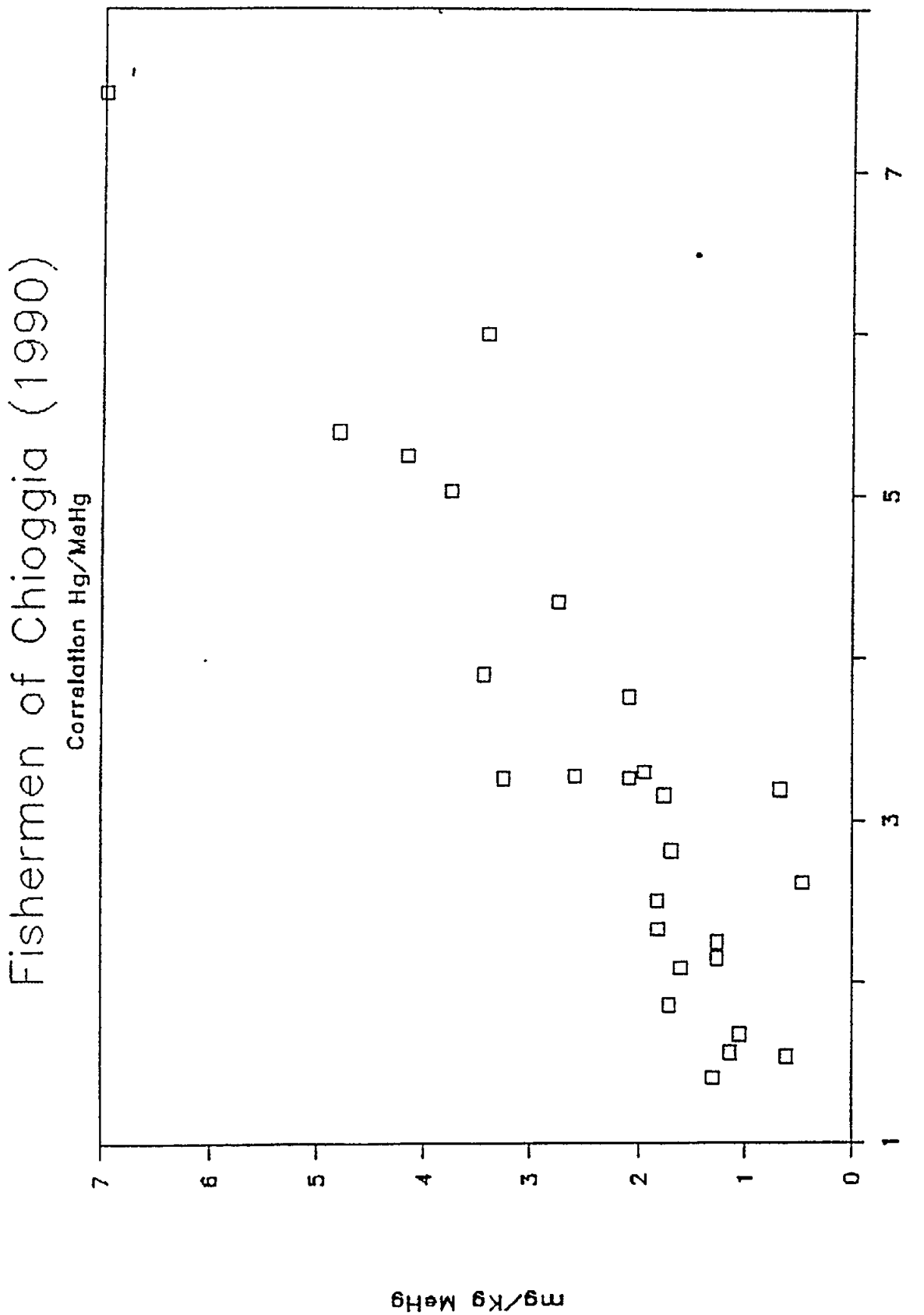

Fishermen of Chioggia (1990)
Correlation Hg/MeHg
mg/Kg MeHg
mg/Kg Hg
0
1
2
3
4
5
6
7
1
3
5
7

Figure 5.

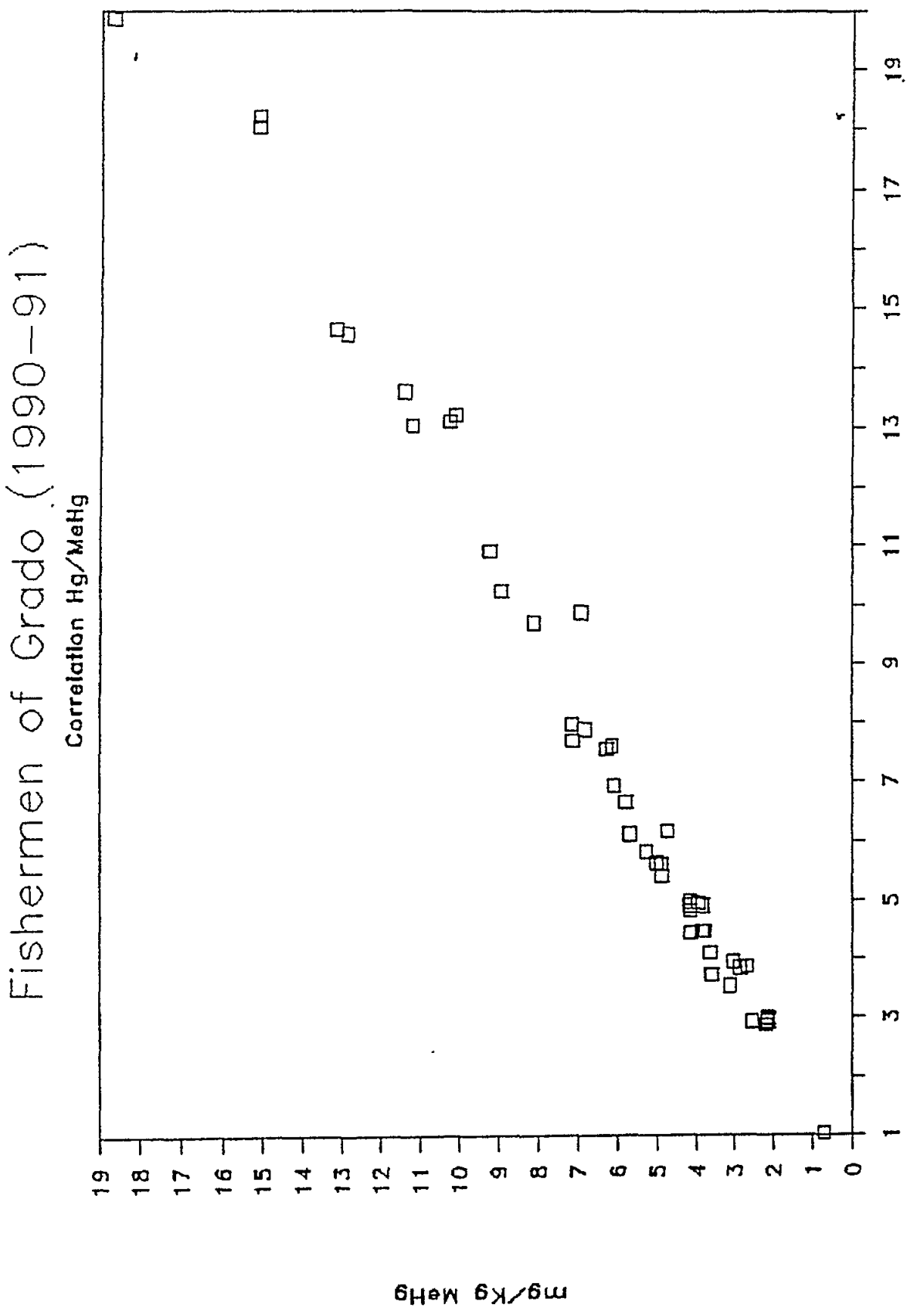
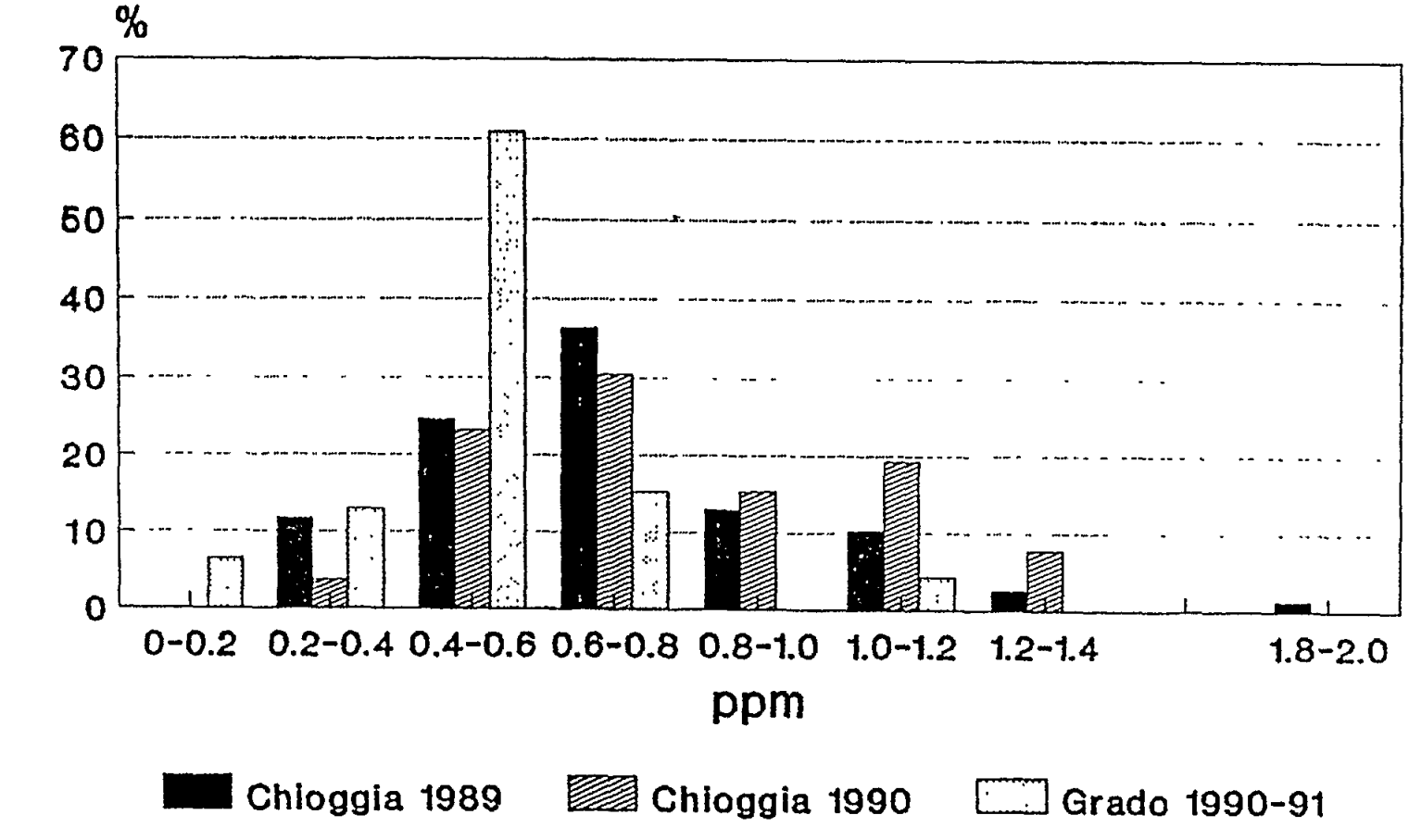
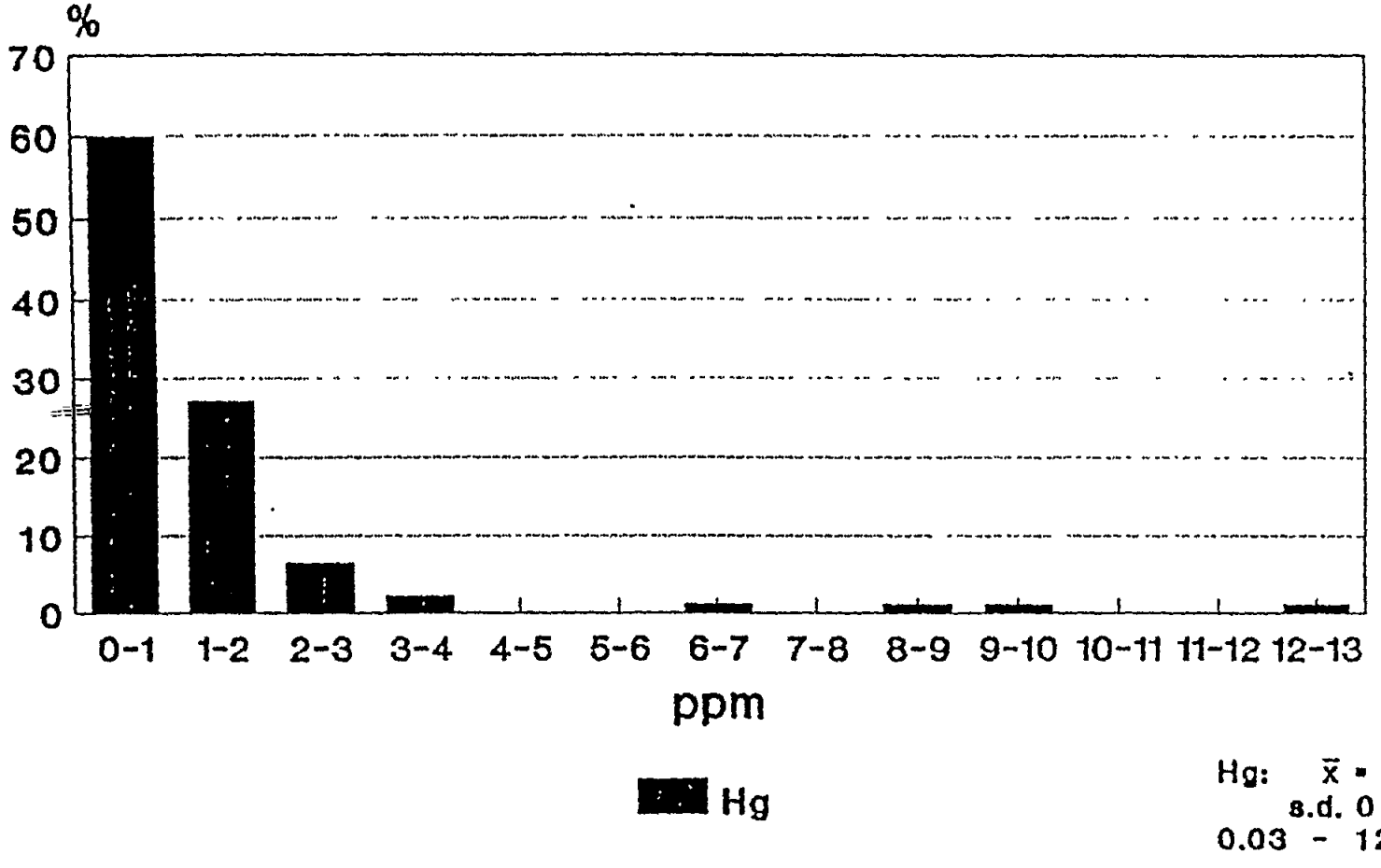

Fishermen of Grado (1990–91)
Correlation Hg/MeHg
mg/Kg MeHg
mg/Kg Hg
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
1
3
5
7
9
11
13
15
17
19

Figure 6.



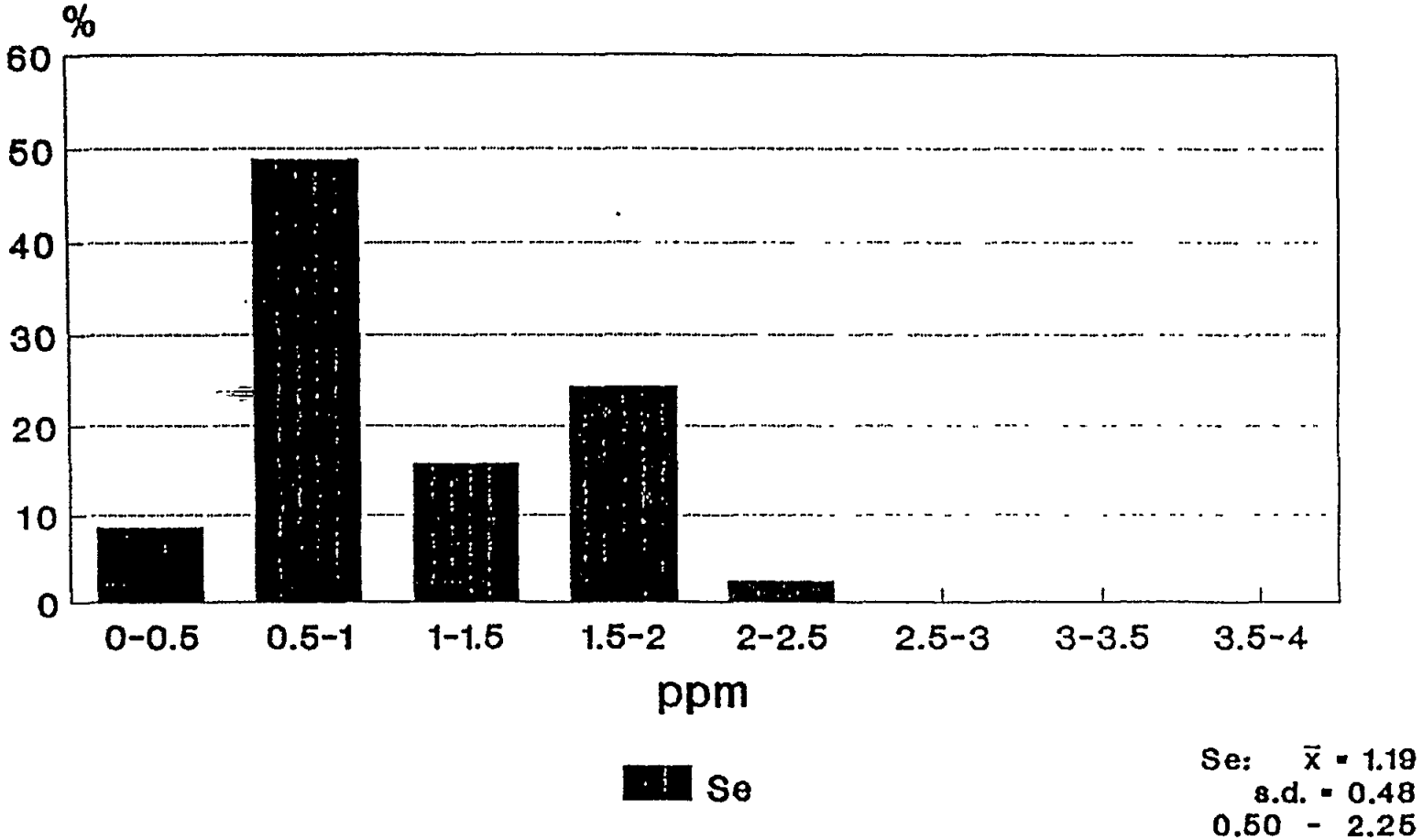
Chioggia 1989-90, Grado 1990-91

Figure 7.



A mountain control population

Figure 8.



A mountain control population

Figure 9.

EVALUATION OF GENOTOXICITY OF METHYLMERCURY BY EXAMINATION OF SISTER CHROMATID EXCHANGES, MICRONUCLEI AND CHROMOSOMAL ABERRATIONS IN FISHERMEN AT RISK IN THE TYRRHENIAN SEA

by

E. Franchi and A. Renzoni

ABSTRACT

The genotoxicity of methylmercury was evaluated by means of sister chromatid exchanges (SCE), chromosomal aberrations (CA) and micronuclei (MN) frequencies in peripheral blood lymphocytes of a population of fishermen, exposed to this metal through seafood consumption. Data on total mercury and methylmercury concentrations in the blood of donors are reported. Mercury levels in blood were correlated with SCE, CA and MN frequencies in order to assess if the exposure to the metal could induce DNA damaging effects. Mercury caused a dose-dependent increase in MN and CA frequencies, but not in SCE frequencies.

1. INTRODUCTION

In the last decades, high mercury levels have repeatedly been reported in marine organisms from the Tyrrhenian Sea and, in general, mercury concentrations in most Mediterranean marine organisms are higher than those found in the same species from other seas (Baldi *et al.*, 1979; Renzoni *et al.* 1979; Leonzio *et al.*, 1981; Barghigiani *et al.*, 1986; UNEP/FAO/WHO, 1987).

Several hypothesis have been formulated to explain this phenomenon:

- the geochemical anomaly of the whole Mediterranean basin, located on a "mercury belt" (Jonasson and Boyle, 1971);
- the thermic conditions of the Mediterranean Sea (temperature higher than 13EC regardless of depth or seasons) that increase, in sediments, the methylation rate resulting in an increased methylmercury bio-availability for water organisms (Bacci, 1989);
- the relative small size of this basin and the slow exchanges of its waters with the ocean.

Anthropogenic sources, from the coastal area of the Tyrrhenian Sea, do not have a great influence on the whole system, even though they may cause heavy local contamination episodes (Bernhard and Renzoni, 1977; Bacci *et al.*, 1979).

The human population living in this area and consuming large amounts of seafood, are exposed to a toxicological hazard. The hazard consists in long-term intake of mercury-rich seafood and concerns mainly fishermen, fish vendors and their families.

Mercury found in marine seafood is mainly in an organic form: methylmercury, an extremely toxic compound for living organisms. The main target of methylmercury, assumed through diet, is the brain. Methylmercury intake resulting in body burdens of less than 0.5 mg/kg body weight, do not usually give rise to detectable neurological signs in adults and corresponds to values of less than 200 µg/R in blood and 50 µg/g in hair (WHO, 1976; WHO, 1990).

In fishermen consuming Mediterranean seafood 4 or more times/week, elevated blood and hair mercury concentrations have been found, sometimes above 200 µg/R and 50 µg/g respectively (Renzoni, 1987; Franchi *et al.*, 1994).

To evaluate the genotoxicity of methylmercury, a group of fishermen, exposed through eating contaminated seafood from the Northern Tyrrhenian Sea, underwent cytogenetic monitoring. The research project consists of the evaluation of the frequency of micronuclei (MN), chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in cell cultures of peripheral blood lymphocytes of fishermen.

Despite its well-known neurotoxicity and teratogenicity (Harada, 1978; Leonard *et al.*, 1983), the genotoxic effects of mercury on humans are not yet completely defined.

Previously published data concerning human populations exposed to mercury through different pathways (food consumption or occupationally) considered mainly chromosomal aberrations induced by this metal (Skerfving, 1970; Skerfving *et al.*, 1974; Verschaeve *et al.*, 1978; Verschaeve *et al.*, 1979; Popescu *et al.*, 1979; Monsalve and Chiappe, 1987; Wagida and Gabal, 1991). Very little data can be found regarding micronuclei (Wagida and Gabal, 1991) and SCE induction (Wulf *et al.*, 1986; Monsalve and Chiappe, 1987).

2. MATERIALS AND METHODS

Blood samples were collected in lithium heparin tubes at the local health centre and then brought to the laboratory within 24 hours.

For each individual a complete questionnaire of about 50 questions was filled in, giving information about age, seafood-based meals, and life habits like smoking and alcohol, which could be confounding factors for cytogenetic analysis (Carrano and Natarajan, 1988).

The total amount of blood, about 10 mR, was divided into two aliquots, one used for cytogenetic analyses (MN, SCE and CA), the other for mercury and methylmercury detection.

2.1 Micronucleus Assay

For each person 2 lymphocyte cultures were set up by adding 0.3 mR of heparinized whole blood to 4.7 mR of complete culture media, consisting of Ham's F10 medium (Flow Laboratories) supplemented with 10% foetal bovine serum (Flow Laboratories), with 1.5% phytohemagglutinin (PHA, Wellcome) and antibiotics (100 IU/mR penicillin and 100 µg/mR streptomycin, Sigma). The cultures were grown at 37EC.

Cytochalasin B (Cyt B, Sigma) was added to each culture after 44 hrs (final concentration 3 µg/mR).

After an incubation time of 72 hrs, cultures were harvested and centrifuged at 1000 rpm for 10 min. The cells were then treated twice with a buffer (NH_4HCO_3 0.9 mM, NH_4Cl 131 mM) for 20 min at 37EC, and centrifuged at 3000 rpm for 15 min. After this treatment they were twice resuspended in cold fixative (glacial acetic/methanol, 3:1) for 20 min at room temperature and centrifuged at 1000 rpm for 10 min. After the final centrifugation cells were dropped onto wet clean slides, air-dried and stained with Giemsa (Merk, 3% in distilled water) for 10 min. The slides were then rinsed in distilled water, air-dried and mounted in Eukitt.

All slides were coded and read blind. For each person a total of 2000 binucleate cells with preserved cytoplasm were scored at a magnification of 400 X. To evaluate the presence of micronuclei, two observations of 1000 cells were performed by different observers on different slides.

Criteria used for the discrimination of true and false MN were those of Countryman and Heddle (1976).

2.2 Sister Chromatid Exchanges and Chromosomal Aberrations Assays

For each subject 5 cell cultures (2 for SCE and 3 for CA assay) were set up by incubating 0.3 mR of heparinized whole blood in 4.7 mR of complete culture media (Ham's F10 medium supplemented with 10% foetal bovine serum, with 1.5% phytohemagglutinin and 100 IU/mR penicillin plus 100 µg/mR streptomycin).

Cell cultures for SCE assay were supplemented with 5-bromodeoxyuridine (BUdR, Sigma), at the final concentration of 9 µg/mR. Lymphocytes were cultured in the dark for 72 hrs and metaphases were blocked during the last 2 hrs with colchicin (Sigma, 4 µg/mR final concentration).

Colchicin (4 µg/mR) was added also to the cultures for CA assay 2 hrs before harvesting. All the cultures were grown at 37EC.

After incubation time (72 and 48 hrs respectively for SCE and CA assays), cultures were harvested and centrifuged (1000 rpm for 10 min.); the supernatant was removed and a hypotonic solution (0.075 M KCl) was added at 37EC for 20 min. After this treatment, cells were centrifuged and fixed twice with cold fixative (glacial acetic acid/methanol, 3:1) for 20 min at room temperature and centrifuged at 1000 rpm for 10 min. After the final centrifugation, cell suspensions were dropped onto wet clean slides, air-dried and stained.

Metaphases for CA were stained with Giemsa (Merk, 3% in distilled water) for 10 min. The slides were then rinsed in distilled water, air-dried and mounted in Eukitt. All slides were coded and analyzed blindly. A total of 100 metaphases for each subject were analyzed by direct microscope examination (1000 X); only cells with 46 chromosomes were considered. Both structural (CA) and numerical (AN) chromosomal aberrations were scored. CA were recorded as chromatid and chromosome aberration and classified as gaps (G), breaks (B), fragments (F) and exchanges (E).

SCE staining was performed using a modification of fluorescence-plus-Giemsa method (Perry and Wolff, 1974). Slides were maintained in the dark, in a solution of 0.5 µg Hoechst 33259 per mR in Sorensen Buffer pH 7.4 for 15 min. They were then washed and placed, face up and completely covered, in SSC solution (NaCl 0.15 M and $Na_3C_6H_5O_7$ 0.015 M). The

submerged slides were exposed to a 300 Watt long wave light, at a distance of 10 cm for 30 min. Finally, slides were washed and stained with Giemsa (Merk, 3% in distilled water) for 10 min. Preparations were mounted in Eukitt.

SCE were analyzed in 40 second-division cells for each person, at magnification of 1000 X. Two observations of 20 cells were performed by different observers on different slides. SCE frequency was calculated as mean number of exchanges per cell and standard deviation.

2.3 Total Mercury Analysis

About 1 mR of blood was treated with nitric acid in decomposition vessels of teflon under pressure (3000kPa) at 120EC for 6-8 hrs (Stöeppler and Backhaus, 1978). The mineralized solution obtained was analyzed by Atomic Absorption Spectrophotometry (Perkin-Elmer mod.2280) using the vapour stream system for mercury. Precision was measured on 6 homogeneous replicates of the same sample and then the coefficient of variation was calculated; the accuracy was tested with certificate standards (NBS, IAEA).

2.4 Methylmercury Analysis

About 1 mR of blood was hydrolyzed with an acid solution; methylmercury was then extracted into an organic solvent, purified by the addition of cysteine solution, re-extracted into organic solvent and then measured by gas chromatography (Horvat *et al.*, 1990). A Gas Chromatograph (Perkin-Elmer F-22) equipped with electron capture detector and a 1m x 2mm borosilicate gas-column packed with 5% Carbowax 20 M on 100/120 Supelcoport was used. The carrier gas is argon/methane (96/5), flows 90 and 40 (as scavenger) mR/min; injector, oven and detector temperatures are 190E, 180E and 280EC respectively. Recovery was calculated by addition method. Precision was measured on 6 homogeneous replicates of the same sample and then the coefficient of variation was calculated; the accuracy was tested through intercalibration exercises and reference materials.

2.5 Statistical Analysis

All data were processed by parametric statistical analysis, using Statgraph (Statistical Graphics Corporation) software on an IBM personal computer.

3. RESULTS AND DISCUSSION

A total of 74 fishermen were examined for total mercury concentration in blood. The age of fishermen ranged from 16 to 75 years. The average of blood mercury levels was 88.84 µg/R ±52.39 standard deviation (S.D.), with a range from 10.08 to 304.11 mg/R fresh weight (f.w.).

Methylmercury determination was performed in 20 samples chosen between those previously analyzed for total mercury. The results (Fig. 1) indicate that most of the metal is in organic form, especially when the concentration of total mercury is high. The x axis intercept (3.5 µg/R f.w.; standard error on the x axis 17.95 µg/R) indicates a background level for inorganic mercury of 20 µg/R. On the basis of the slope y = -3.5 + 0.959x found in the methylmercury

experiment, its concentration in all samples was calculated. In Table 1 beside age, total mercury and methylmercury levels are reported.

Cell cultures for 63 donors were set up to perform MN assay. In Table 2 all parameters considered were reported : age, blood mercury levels, MN frequencies, smoking, and seafood consumption. The age range was from 16 to 75 years, and the average seafood based meals/week was 7.28 ± 3.40 S.D. Blood mercury levels ranged from 10.08 to 304.11 µg/ℝ with a mean of 88.97 ± 54.08 S.D. The average frequency of micronucleated lymphocytes was 8.74 ‰ ± 2.56 S.D. When data were analyzed with linear regression analysis, a significant correlation was found between micronucleus frequency and blood mercury levels ($p<0.001$)(Fig.2). The frequency of micronuclei also showed, in linear regression analysis, a significant correlation with the age of each subject ($p<0.005$) (Fig.3); no correlation was found with seafood meals.

Since the correlation between MN frequency vs. age of subjects and between Hg levels vs. age were found positive ($p<0.001$ and $p<0.005$, respectively), to evaluate the effective correlation between MN frequency and mercury exposure, MN frequencies and Hg levels adjusted for age were correlated. Even in this case, a positive linear regression between MN frequencies and Hg concentrations in blood was found ($F=16.79$; $p<0.0005$). All the data were log-transformed.

Chromosomal aberration frequencies were evaluated on 59 samples. In Table 3 structural chromosomal aberrations are reported: (CA) classified as gaps (G), breaks (B), and other, and numerical chromosomal aberrations (AN) divided per number of chromosomes per cell. In Table 3 age, blood mercury levels, the total number of chromosomal aberrations (CA%), chromosomal aberrations included gaps (AC + GAP%) and the number of aneuploid cells (AN%) are reported. A statistical correlation was found between mercury concentration in blood and number of chromosomal aberrations (AC%)($p<0.05$); the correlation was even more significant when gaps were also included ($p=0.005$) (Fig.4). Aneuploidy did not correlate with the parameters taken into account.

In Table 5 data regarding age, smoking habit, mercury levels, and SCE (average and S.D.) in 25 samples are reported. SCE frequencies, analyzed in regression analysis, did not show any significant correlation with mercury exposure even considering the incidence of smoking habit of the donors. Donors were divided into 3 groups: No. 1 composed by no smokers, No. 2 smokers, and No. 3 ex-smokers. Variance analysis revealed an increase in SCE frequency between non-smoker and smoker groups, while the frequency was almost the same between non-smoker and ex-smoker donors (Fig.5). These results could confirm that SCE assay is more suitable to reveal recent DNA-damages.

4. CONCLUSION

In this group of fishermen the exposure is due to a long-term intake of mercury-rich seafood; furthermore, as the intake of contaminated food is rather constant, it is assumed that the steady state is reached and blood mercury levels do not vary consistently. Blood is largely disponible, suitable for setting up cell cultures, and therefore can be considered a useful tissue to evaluate mercury exposure.

Our findings show an increase in cytogenetic damage in peripheral blood lymphocytes of fishermen exposed to mercury through seafood consumption.

When the data were processed by regression analysis a positive correlation was found between total mercury concentration in blood and micronucleus frequency ($p<0.001$) evaluated in peripheral blood lymphocytes. A positive correlation was found between MN frequency and age; this finding is in agreement with other studies (Fenech & Morley, 1986; Sorsa *et al.*, 1988; Migliore *et al.*, 1991) and confirms the importance of this factor in human lymphocyte MN assay. However, the contaminant can be considered the main cause of the increase in micronucleated lymphocytes and confirms the persistence of exposure to mercury through diet.

A linear correlation was also found between mercury concentrations in blood and chromosomal aberrations frequencies ($p<0.05$). This correlation was even more significant when gaps were included ($p<0.005$).

SCE frequencies did not correlate with mercury levels in blood; this could be explained by the long term exposure to mercury in this group of fishermen, while SCE assay has been considered to be more suitable in assessing short-term exposure. Variance analysis showed the importance of smoking habits to the increase in the number of exchanges per cell.

Another aim of our project was also to perform a cytogenetic surveillance of the monitored population, in order to assess the sensitivity of this technique at environmental levels of exposure.

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Table 1

Data concerning age, total mercury (Hg) and methylmercury (MeHg) concentrations

Code (Individuals)	Age (Years)	Hg µg/ℝ f.w.	MeHg µg/ℝ f.w.	Code (Individuals)	Age (Years)	Hg µg/ℝ f.w.	MeHg µg/ℝ f.w.
A	25	44.58	39.25	AE	37	101.19	93.54
B	26	135.08	126.04	AF	35	74.08	67.54
C	59	93.78	86.43	AG	50	119.36	110.97
D	45	52.69	47.03	AH	33	91.12	83.88
E	59	153.59	143.79	AH	33	63.28	57.18
E	60	133.81	124.82	AI	39	133.69	124.71
F	50	43.35	38.07	AL	57	156.16	146.26
F	51	86.64	79.59	AL	58	151.31	141.61
G	52	52.02	46.39	AM	42	137.26	128.13
G	53	106.57	98.70	AM	43	88.01	80.90
H	74	89.97	82.78	AN	59	118.39	110.04
H	75	117.37	109.06	AN	60	112.10	104.00
I	57	87.93	80.82	AO	35	67.94	61.65
I	58	66.43	60.21	AO	36	77.51	70.83
L	27	31.51	26.72	AO	50	83.52	76.59
M	29	28.66	23.98	AQ	49	49.79	44.25
N	39	43.61	38.32	AQ	50	34.22	29.32
O	65	54.78	49.03	AR	50	44.18	38.87
P	69	49.77	44.23	AS	52	70.23	63.85
Q	65	29.86	25.13	AT	35	96.39	88.94
R	24	68.73	62.41	AU	28	29.32	24.62
S	51	89.72	82.54	AU	29	141.44	132.14
S	52	119.23	110.84	AV	49	83.55	76.62
T	45	100.24	92.63	AZ	28	43.61	38.32
T	46	131.79	122.88	BA	45	169.89	159.42
U	40	50.13	44.57	BA	46	179.35	168.50
U	41	68.48	62.17	BB	22	10.08	6.17
V	45	126.72	118.02	BC	51	45.72	40.34
Z	41	37.55	32.51	BC	51	27.25	22.63
AA	42	90.34	83.13	BD	53	61.41	55.39
AA	43	66.04	59.83	BE	57	60.37	54.39
AB	54	100.27	92.66	BF	55	190.75	179.42
AB	54	78.08	71.38	BG	55	304.11	288.14
AC	45	27.49	22.86	BH	22	35.08	30.14
AD	44	137.51	128.37	BI	26	48.33	42.85
AD	44	118.20	109.85	BL	20	32.30	27.47
AE	36	252.25	238.41	BM	16	76.86	70.21

Table 2

Data concerning age, blood mercury levels (Hg), micronucleus frequency (MN ‰), seafood consumption, smoking habit (S=smoker, NS=no smoker)

Code (Individuals)	Age (Years)	Hg µg/ℝ f.w.	MN‰	Seafood meals/week	Smoking habit
A	25	44.58	5.5	5	S
B	26	135.08	7.5	2	NS
C	59	93.78	12.0	7	NS
D	45	52.69	5.0	5	NS
E	59	153.59	12.0	10	NS
G	52	52.02	7.5	10	S
H	74	89.97	7.0	4	NS
L	27	31.51	9.0	6	S
M	29	28.66	8.0	8	S
N	39	43.61	9.0	6	S
O	65	54.78	7.0	5	S
P	69	49.77	8.5	2	S
Q	65	29.86	6.5	2	NS
R	24	68.73	7.0	4	NS
S	51	89.72	6.5	7	NS
T	45	100.24	8.5	7	S
U	40	50.13	10.0	7	S
V	45	126.72	8.0	13	NS
AA	42	90.34	9.5	6	NS
AB	54	100.27	11.0	14	S
AC	45	27.49	11.5	2	S
AD	44	137.51	10.5	1	S
AE	36	252.25	9.5	11	NS
AF	35	74.08	13.5	12	S
AG	50	119.36	11.5	7	S
AH	33	91.12	10.5	12	S
AI	39	133.69	10.0	7	S
AR	50	44.18	10.0	1	S
AS	52	70.23	10.0	7	NS
AT	35	96.39	9.0	10	S

Code (Individuals)	Age (Years)	Hg µg/ℝ f.w.	MN‰	Seafood meals/week	Smoking habit
AU	28	29.32	4.5	7	NS
AV	49	83.55	7.0	7	NS
AZ	28	43.61	9.0	12	NS
BA	45	169.89	11.5	5	NS
BB	22	10.08	1.5	2	NS
E	60	133.81	9	10	NS
F	51	86.64	12.0	7	S
G	53	106.57	8.5	10	S
H	75	117.37	7.5	3	NS
I	58	66.43	11.0	7	NS
S	52	119.23	9.0	7	NS
T	46	131.79	7.0	10	S
U	41	68.48	10.5	7	S
AA	43	66.04	11.5	4	NS
AB	54	78.08	8.0	14	S
AD	44	118.20	8.5	10	S
AE	37	101.19	7.5	2	NS
AH	33	63.28	8.0	10	S
AL	57	156.16	9.0	14	NS
AM	43	88.01	7.0	7	S
AN	60	112.10	12.5	12	NS
AO	36	77.51	8.5	12	NS
AQ	50	34.22	14.5	7	NS
BA	46	179.35	13.5	5	NS
BC	51	27.25	6.5	5	NS
BD	53	61.41	6.5	10	NS
BE	57	60.37	5.0	5	NS
BF	55	190.75	10.0	10	NS
BG	55	304.11	13.0	7	NS
BH	22	35.08	5.0	1	NS
BI	26	48.33	6.5	7	S
BL	20	32.30	3.5	6	NS
BM	16	76.86	7.0	7	NS

Table 3

Data concerning age, blood mercury levels (Hg), total number of scored cells per person, chromosomal aberrations classified as gas (G), breaks (B) and other, numerical chromosomal aberrations divided per number of chromosomes per cell

Code individuals	Age (years)	Hg µg/ℝ f.w.	Scored cells	Chromosomal Aberrations				Cells with chromosome numbers			
				G	B	Other	Total	<46	47	>47	Total
A	25	44.58	100	1	3	0	4	0	0	0	0
B	26	135.08	100	0	1	0	1	0	0	0	0
C	59	93.78	100	1	2	2	5	2	0	0	2
D	45	52.69	100	0	2	0	2	0	0	0	0
E	59	153.59	100	9	6	1	16	0	0	1	1
F	50	43.35	100	2	2	1	5	0	0	0	0
G	52	52.02	100	3	0	0	3	0	0	0	0
H	74	89.97	100	2	0	0	2	0	3	0	3
I	57	87.93	100	2	1	1	4	0	1	0	1
L	27	31.51	100	2	1	0	3	0	1	1	2
M	29	28.66	100	2	2	0	4	0	0	0	0
N	39	43.61	100	1	0	3	4	0	0	0	0
O	65	54.78	100	2	0	0	2	0	3	0	3
P	69	49.77	100	1	1	0	2	0	0	0	0
Q	65	29.86	100	2	2	1	5	0	0	0	0
R	24	68.73	100	1	0	0	1	0	0	0	0
S	51	89.72	100	3	2	1	6	0	1	1	2
T	45	100.24	100	1	0	0	1	0	2	0	2
U	40	50.13	100	1	0	0	1	0	1	0	1
V	45	126.72	100	1	1	1	3	0	3	0	3
AA	42	90.34	100	1	0	0	1	0	0	1	1
AC	45	27.49	100	0	1	0	1	0	0	0	0
AF	35	74.08	100	0	1	0	1	0	0	0	0
AI	39	133.69	100	0	0	0	0	0	2	1	3
AL	57	156.16	100	5	0	1	6	0	1	1	2
AM	42	137.26	100	1	2	0	3	0	2	0	2
AN	59	118.39	100	1	0	0	1	0	0	0	0
AO	35	67.94	100	2	0	0	2	0	0	0	0
AQ	49	49.79	100	1	1	2	4	0	0	0	0

Code individuals	Age (years)	Hg µg/ℝ f.w.	Scored cells	Chromosomal Aberrations				Cells with chromosome numbers			
				G	B	Other	Total	<46	47	>47	Total
AR	50	44.18	100	1	0	0	1	0	0	0	0
AT	35	96.39	100	1	1	0	2	0	0	0	0
AU	28	29.32	100	1	0	0	1	0	1	0	1
AV	49	83.55	100	1	1	1	3	0	0	0	0
F	51	86.64	100	5	3	0	8	0	1	0	1
G	53	106.57	100	5	3	0	8	0	0	0	0
H	75	117.37	100	3	1	1	5	0	0	0	01
I	58	66.43	100	2	1	1	4	0	1	0	1
S	52	119.23	100	3	0	0	3	0	1	0	1
T	46	131.79	100	7	2	0	9	0	0	0	0
U	41	68.48	100	3	2	0	5	0	0	0	0
AA	43	66.04	100	2	5	0	7	0	1	0	1
AB	54	78.08	100	1	1	0	2	0	0	0	0
AD	44	118.20	100	5	2	0	7	0	2	0	2
AE	37	101.19	100	0	4	0	4	0	11	0	11
AH	33	63.28	100	1	2	0	3	0	0	0	0
AM	43	88.01	100	4	0	0	4	0	0	0	0
AN	60	112.10	100	2	3	1	6	0	0	0	0
AO	36	77.51	100	0	0	2	2	0	0	0	0
AQ	50	34.22	100	1	2	0	3	0	0	0	0
BA	46	179.35	100	5	2	0	7	0	0	1	1
BC	51	27.25	100	0	1	0	1	0	0	0	0
BD	53	61.41	100	0	1	0	1	0	0	0	0
BE	57	60.37	100	1	2	0	3	0	0	0	0
BF	55	190.75	100	1	2	0	3	0	2	0	2
BG	55	304.11	100	2	2	2	6	0	0	0	0
BH	22	35.08	100	2	1	0	3	0	0	0	0
BI	26	48.33	100	3	0	0	3	0	0	0	0
BL	20	32.30	100	2	0	0	2	0	0	0	0
BM	16	76.86	100	2	2	0	4	0	0	0	0

Table 4

Data concerning age, blood mercury levels (Hg), chromosomal aberrations frequency (AC%), chromosomal aberrations frequency included gaps (AC+GAP%) and aneuploid cells (ANEUPL%)(100 scored cells per person)

Code (individuals)	Age (Years)	Hg µg/ℝ	AC%	AC+GAP%	ANEUPL%
A	25	44.58	3	4	0
B	26	135.08	1	1	0
C	59	93.78	4	5	2
D	45	52.69	2	2	0
E	59	153.59	7	16	1
F	50	43.35	3	5	0
G	52	52.02	0	3	0
H	74	89.97	0	2	3
I	57	87.93	2	4	1
L	27	31.51	1	3	2
M	29	28.66	2	4	0
N	39	43.61	3	4	0
O	65	29.86	3	5	0
P	69	49.77	1	2	0
Q	65	29.86	3	5	0
R	24	68.73	0	1	0
S	51	89.72	3	6	2
T	45	100.24	0	1	2
U	40	50.13	0	1	1
V	45	126.72	2	3	3
AA	42	90.34	0	1	1
AC	45	27.49	1	1	0
AF	35	74.08	1	1	0
AI	39	133.69	0	0	3
AL	57	156.16	1	6	2
AM	42	137.26	2	3	2
AN	59	118.39	0	1	0
AO	35	67.94	0	2	0
AQ	49	49.79	3	4	0

Code (individuals)	Age (Years)	Hg µg/ℝ	AC%	AC+GAP%	ANEUPL%
AR	50	44.18	0	1	0
AT	35	96.39	1	2	0
AU	28	29.32	0	1	1
AV	49	83.55	2	3	0
F	51	86.64	3	8	1
G	53	106.57	3	8	0
H	75	117.37	2	5	1
I	58	66.43	2	4	1
S	52	119.23	0	3	1
T	46	131.79	2	9	0
U	41	68.48	2	5	0
AA	43	66.04	5	7	1
AB	54	78.08	1	2	0
AD	44	118.20	2	7	2
AE	36	101.19	4	4	11
AH	33	63.28	2	3	0
AM	43	88.01	0	4	0
AN	60	112.10	4	6	0
AO	36	77.51	2	2	0
AQ	50	34.22	2	3	0
BA	46	179.35	2	7	0
BC	51	27.25	1	1	0
BD	53	61.41	1	1	0
BE	57	60.37	2	3	0
BF	55	190.75	2	3	2
BG	55	304.11	4	6	0
BH	22	35.08	1	3	0
BI	26	48.33	0	3	0
BL	20	32.30	0	2	0
BM	16	76.87	2	4	0

Table 5

Data concerning age, total mercury concentration (Hg), smoking (S= smoker, NS= no-smoker, EX S+ ex-smoker) sister chromatid exchanges average (expressed as number of exchanges per cell) and standard deviation (S.D.)

Code (Individuals)	Age (years)	Smoking habit	Hg-tot µg/R f.w.	SCE	
				Average	S.D.
F	51	S	86.64	7.68	2.69
G	53	S	106.57	8.84	3.29
H	75	NS	117.37	6.88	3.32
I	58	NS	66.43	7.46	3.02
S	52	NS	119.23	9.12	3.11
U	41	EX S	68.48	9.28	3.61
AA	43	EX S	66.04	8.66	3.38
AB	54	S	78.08	8.84	2.20
AD	44	S	118.20	8.20	2.82
AE	37	EX S	101.19	6.64	1.93
AH	33	S	63.28	9.04	4.02
AL	57	EX S	156.16	7.48	3.25
AM	43	S	88.01	9.14	3.90
AN	60	EX S	112.10	6.82	2.64
AO	36	NS	77.51	7.68	3.15
AQ	50	EX S	34.22	7.92	3.11
BA	46	EX S	179.35	6.80	3.69
BC	51	NS	27.25	8.16	3.51
BD	53	NS	61.41	7.70	3.29
BE	57	EX S	60.37	5.90	2.31
BG	52	EX S	304.11	6.88	2.59
BH	22	NS	35.08	5.68	2.78
BI	26	S	48.33	6.82	2.80
BL	20	NS	32.30	6.98	2.62
BM	16	NS	76.86	6.60	3.00

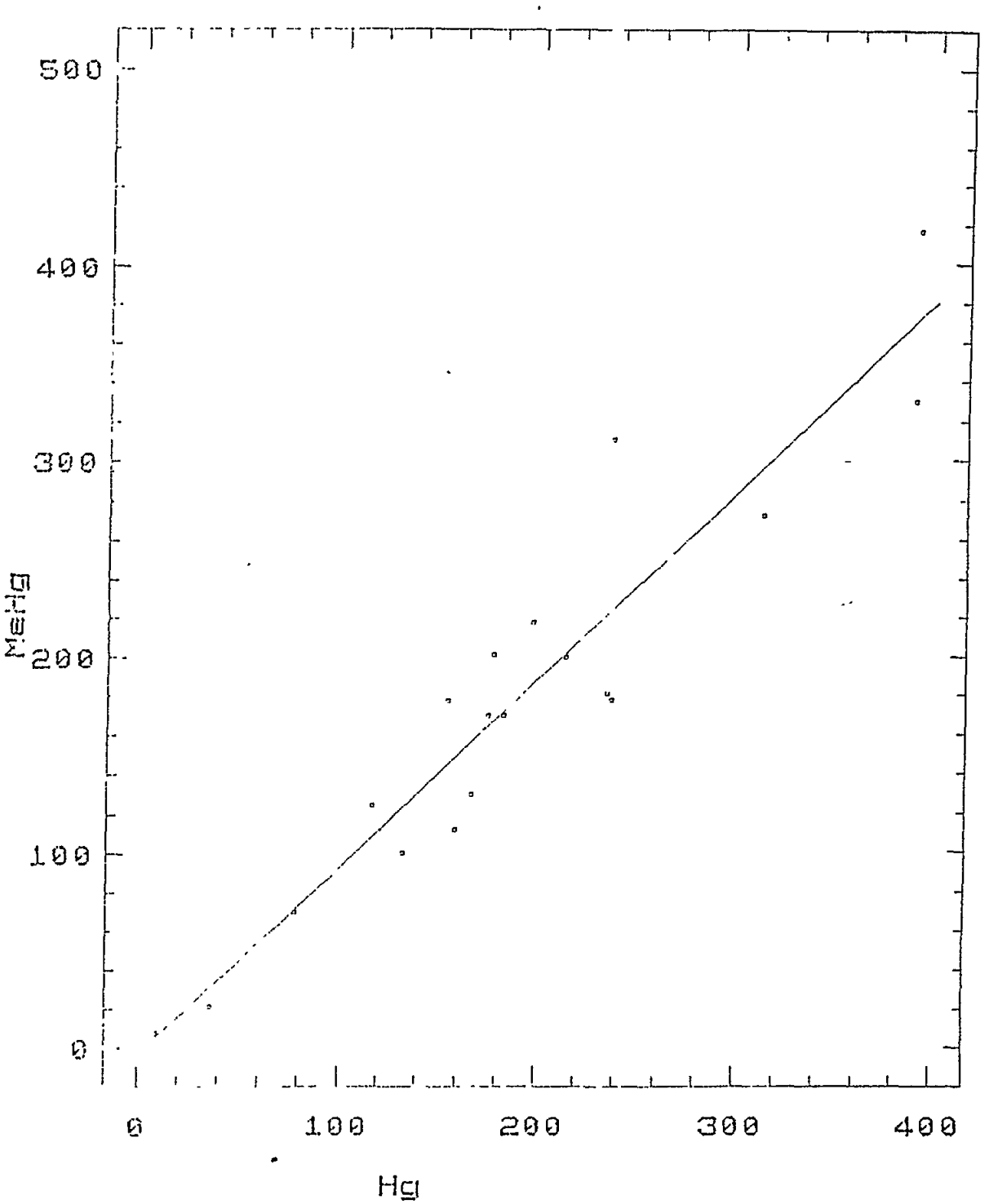

Figure 1. Linear regression analysis between total mercury (Hg) and methylmercury (MeHg) expressed on µg/ℓ f.w.

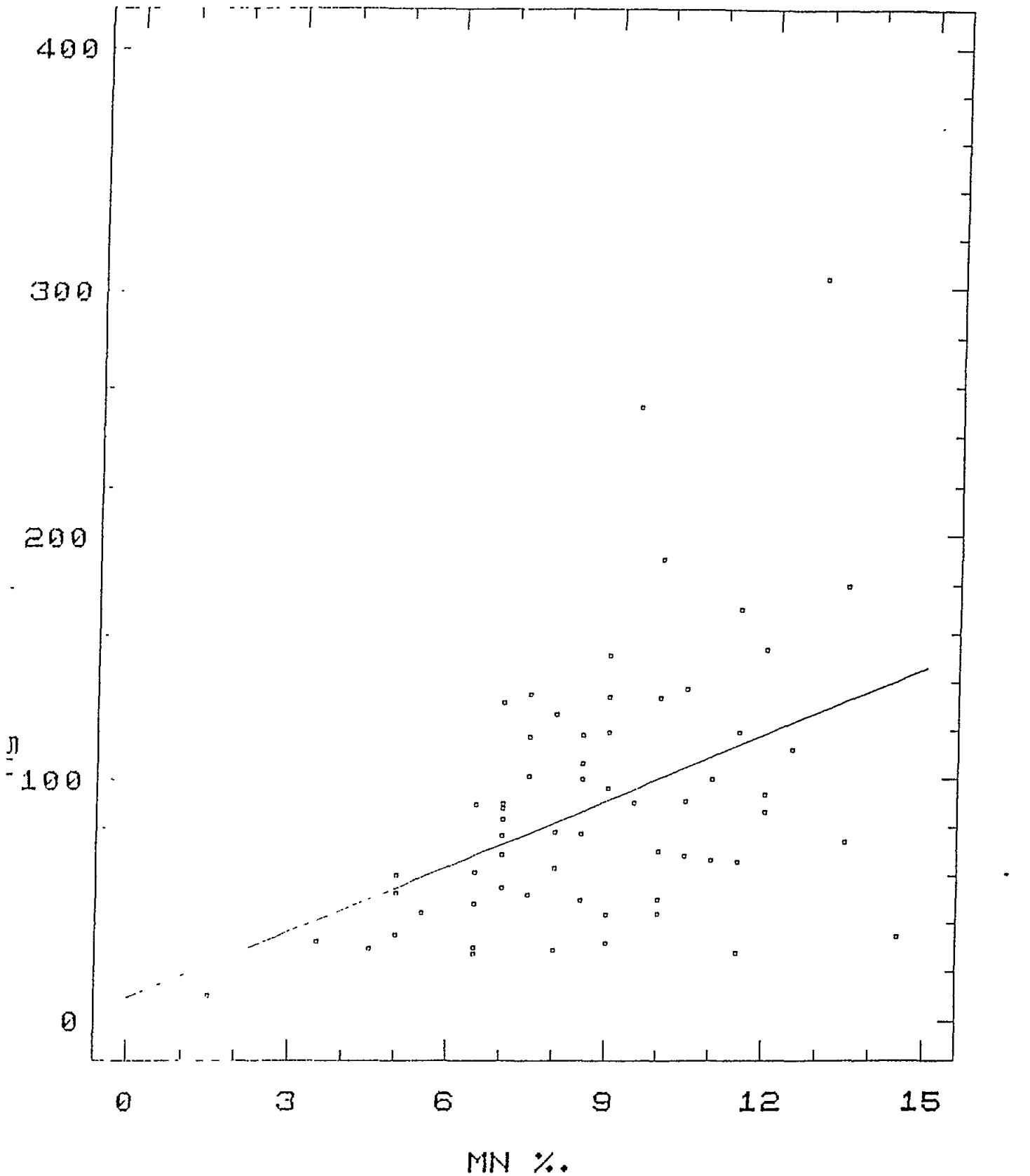

Figure 2. Regression analysis between micronucleus frequencies (MN‰) and mercury concentrations in blood (Hg µg/ℓ f.w.) ($p<0.001$)

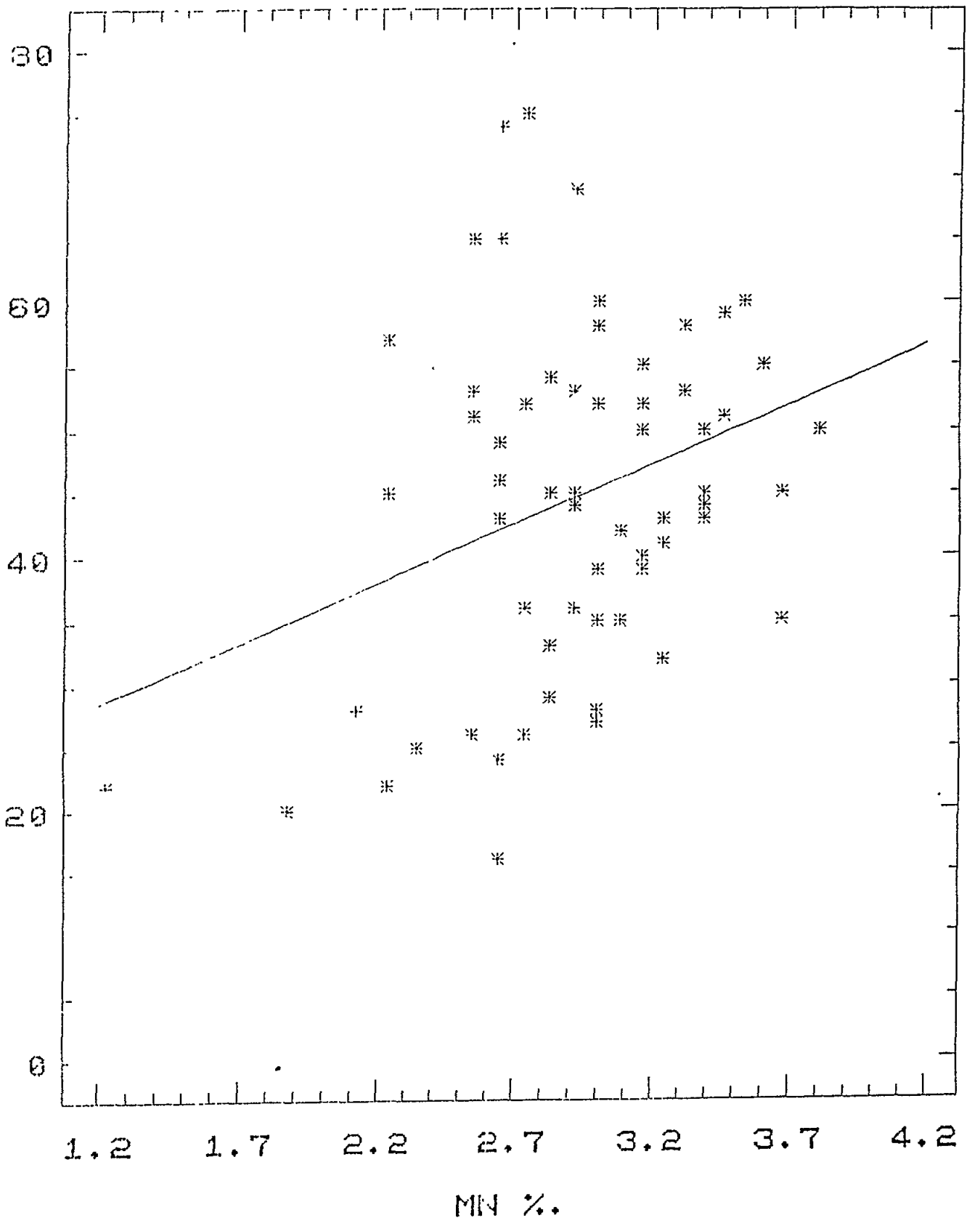

Figure 3. Regression analysis between micronucleated lymphocytes (MN‰) and age of donors

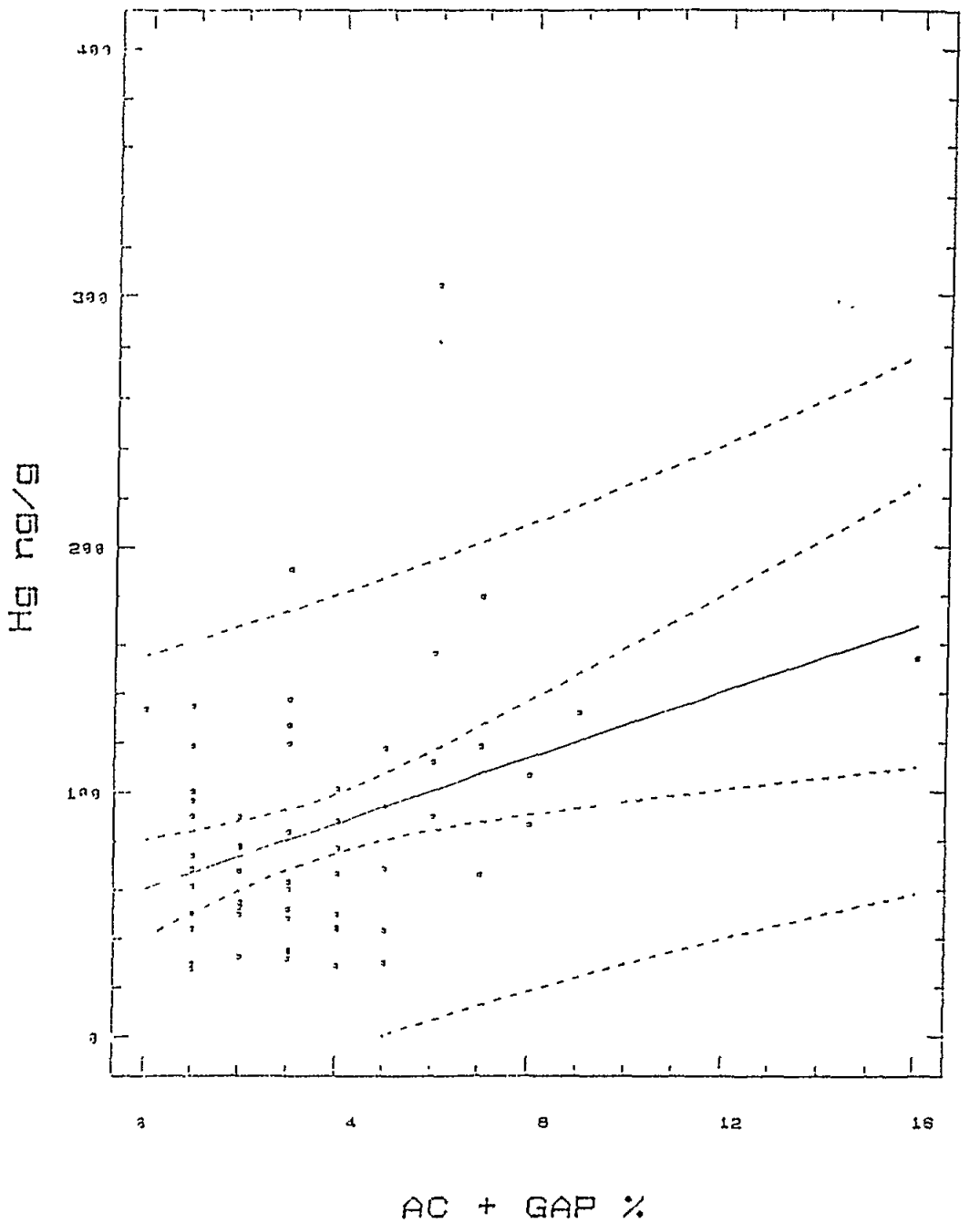

Figure 4. Linear regression analysis between chromosomal aberrations (AC + GAP%) and mercury concentrations in blood (Hg µg/ℓ f.w.) ($p < 0.005$)

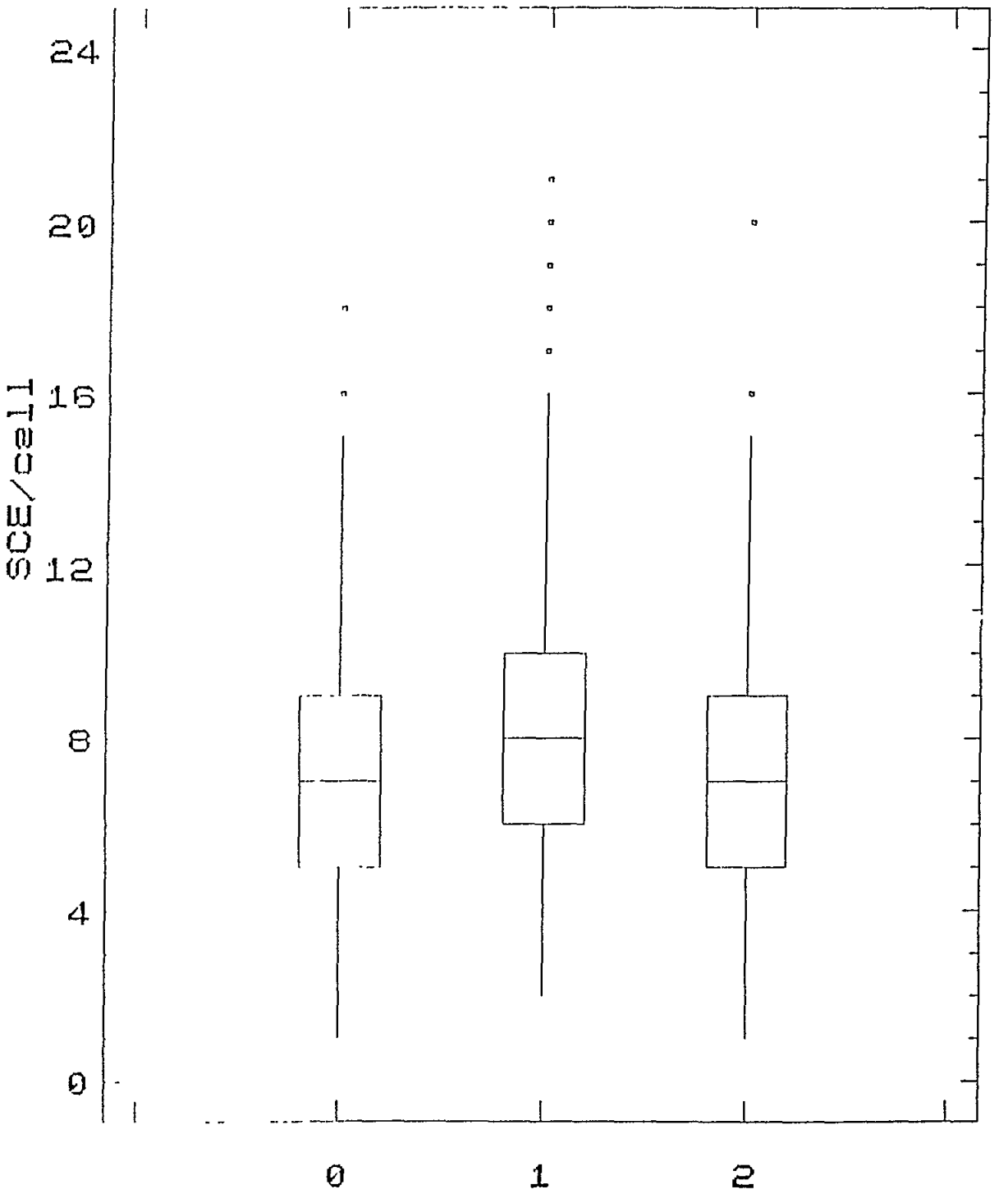

Figure 5. Variance analysis between sister chromatid exchanges frequency (SCE) and smoking habits (0= no smokers; 1 = smokers; 2 = ex smokers)

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