MEDITERRANEAN ACTION PLAN
MED POL

UNITED NATIONS ENVIRONMENT PROGRAMME

WORLD HEALTH ORGANIZATION

DEVELOPMENT AND TESTING OF SAMPLING AND ANALYTICAL TECHNIQUES FOR MONITORING OF MARINE POLLUTANTS (ACTIVITY A)

MISE AU POINT ET ESSAI DES TECHNIQUES D'ECHANTILLONNAGE ET D'ANALYSE POUR LA SURVEILLANCE CONTINUE DES POLLUANTS MARINS (ACTIVITE A)

Final report on project on Control of Intestinal Pathogens (*Campylobacter* and *Aeromonas*) in marine coastal recreational areas

Rapport final sur le projet sur le Contrôle des Agents Pathogènes intestinaux (*Campylobacter* et *Aeromonas*) dans les zones côtières marines à usage récréatif

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

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

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

Cette série permettra de rassembler et de diffuser certains des rapports scientifiques établis dans le cadre de la mise en oeuvre des diverses composantes du PAM: Programme de surveillance continue et de recherche en matière de pollution (MED POP), Plan Bleu, Programme d'actions prioritaires, Aires spécialement protégées et Centre régional pour l'intervention d'urgence contre la pollution marine accidentelle.

GENERAL INTRODUCTION

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

- Integrated planning of the development and management of the resources of the Mediterranean Basin (management component);

- Co-ordinated programme for research, monitoring and exchange of information and assessment of the state of pollution and of protection measures (assessment component);

- Framework convention and related protocols with their technical annexes for the protection of the Mediterranean environment (legal component).

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

MED POL - Phase I (1976-1980)

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

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

- to formulate and carry out a co-ordinated pollution monitoring and research programme taking into account the goals of the Mediterranean Action Plan and the capabilities of the Mediterranean research centres to participate in it;

- to assist national research centres in developing their capabilities to participate in the programme;

- to analyse the sources, amounts, levels, pathways, trends and effects of pollutants relevant to the Mediterranean Sea;

- to provide the scientific/technical information needed by the Governments of the Mediterranean States and the EEC for the negotiation and implementation of the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols;

- to build up consistent time-series of data on the sources, pathways, levels and effects of pollutants in the Mediterranean Sea and thus to contribute to the scientific knowledge of the Mediterranean Sea.

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

MED POL - Phase II (1981-1990)

The Intergovernmental Review Meeting of Mediterranean Coastal States and First Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution, and its related protocols (Geneva, 5-10 February 1989), having examined the status of MED POL - Phase I, recommended that during the 1979/80 biennium a Long-term pollution monitoring and research programme should be formulated.

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

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

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

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

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

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

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

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

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

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

From the tenth volume onwards, the MAP Technical Report Series contains final reports on research projects, assessment documents, and other reports on activities performed within the framework of MED POL-Phase II, as well as documentation originating from other components of the Mediterranean Action Plan.

This seventy fifth volume of the MAP Technical Reports Series contains the final report of a research project on Control of intestinal pathogens (*Campylobacter* and *Aeromonas*) in marine coastal recreational areas, completed within the framework of MED POL in Activity A "Development and testing of sampling and analytical techniques for monitoring of marine pollutants".

INTRODUCTION GENERALE

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

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

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

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

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

MED POL - Phase I (1976-1980)

Le programme coordonné de sruveillance 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.

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

MED POL-Phase II (1981-1990)

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

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

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

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

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

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

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

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

La surveillance continue des polluants affectant le milieu marin de la Méditerranée ainsi que la recherche menée à leur sujet répondent en premier lieu aux prescriptions immédiates et à long terme de la Convention de Barcelone 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.

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

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

A partir du dixième volume, la Série des rapports techniques du PAM, comprend des rapports finaux sur les projets de "recherche", des documents d'évaluation et d'autres rapports d'activités effectués dans le cadre de MED POL-Phase II, ainsi que de la documentation prise dans d'autres domaines du Plan d'action pour la Méditerranée.

Ce soixante quinzième volume de la Série des rapports techniques du PAM comprend le rapport final d'un projet sur le Contrôle des agents pathogènes intestinaux (*Campylobacter* et *Aeromonas*) dans les zones côtières marines à usage récréatif, exécuté dans le cadre de la Phase II du MED POL, dans l'Activité A "Mise au point et essai des techniques d'échantillonnage et d'analyse pour la surveillance continue des polluants marins.

CONTROL OF INTESTINAL PATHOGENS (*Campylobacter* and *Aeromonas*) IN MARINE COASTAL RECREATIONAL AREAS IN VALENCIA, SPAIN

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1. INTRODUCTION

1.1 Injured faecal coliforms

Two methods are specified by the E.E.C. for the microbiological analysis of recreational waters (Directive 76/160), the most probable number (MPN) method and the membrane filter technique (MF). The MF method has gained wide acceptance because the procedure is simple, rapid and precise and gives definitive results.

The methods given as examples vary greatly in relative levels of accuracy when used for counting coliform organisms in polluted seawater (Stanfield *et al.* 1977, Gameson 1981), because factors such as turbidity (Fryt 1979), high number of noncoliform bacteria (Clark 1980), diluents (McFeters *et al.* 1982) and membrane filter type (McFeters and Stuart 1972, Brodsky and Schiemann 1982, Tobin and Dutka 1977, Lorenz and Tuovinen 1979) may influence the sensitivity of the procedure (LeChevallier *et al.* 1983).

In the commonly used MPN method a serious limitation is the time required to complete the testing. The need for rapid determination of the sanitary quality of water has been cited most often in relation to testing water subject to sewage pollution.

In addition none of the recommended methods specify resuscitation (Pike and Ridgway 1985). Recovery of injured organisms on selective media has been shown to be poor, indicating that growth is inhibited (Bisonnette *et al.* 1975) and lower recovery efficiencies of these organisms may be due to the inhibition of biochemical reactions (Olson 1978). Experiments have shown that respiration, glucose transport and ATP levels, all decrease in injured populations (Camper and McFeters 1979); electron microscopy of injured cells has demonstrated changes in the cell membrane (Zaske *et al.* 1980). A number of environmental factor (sunlight, bacteriophages, predators, sedimentation, toxic substances and lack of nutrients) injure or kill coliform bacteria in seawater (Carlucci *et al.* 1959). As a result many investigators have proposed alternative MPN or MF methods (LeChevallier *et al.* 1983, Reasoner *et al.* 1979, Dufour *et al.* 1981, Green *et al.* 1977, Stuart *et al.* 1977). These findings indicate the need to determine injured faecal coliforms in the marine environment.

1.2 Motile *Aeromonas*

Members of the genus *Aeromonas* are now clearly differentiated from members of the *Enterobacteriaceae* and from members of the genera *Pseudomonas* and *Vibrio*; however, *Aeromonas* classification at a specific level is not yet unequivocally established (Popoff 1984) as is indicated by the observation of more than one DNA hybridization group in each of the three defined species (Popoff *et al.* 1981). The DNA hybridization groups within each of the phenotypic groups are separable by using a few biochemical characteristics (Altwegg *et al.* 1990). A number of characteristic properties of motile *Aeromonas* is given in table 1 (Popoff 1984). However, with *Aeromonas* taxonomy currently in a state of flux, having 12 or more DNA-DNA hybridization groups (genotypes) within the genus and 8 proposed and/or recognized species to date, virulence is difficult to assess (Carnahan *et al.* 1990).

Aeromonas species are known for their importance as pathogens in fish, reptiles and warm-blooded animals (Janda and Duffey 1988). In recent years the significance of *Aeromonas* species is receiving increasing attention as human pathogens (Burke *et al.* 1983, 1984). They are considered to be of public health significance when found in large numbers in the environment (Kaaper *et al.* 1981). The aquatic environment is considered the major source of infection (Burke *et al.* 1984, Joseph *et al.* 1979). Reports from many parts of the world suggest that *Aeromonas* species cause an acute self-limiting diarrhoeal illness in man (Barer *et al.* 1986, Mascher *et al.* 1989). *A. hydrophila* and *A. sobria* have been more frequently associated with human infections (Daily *et al.* 1981, Diaz and Velasco 1987) whereas *A. caviae* is less invasive (Watson *et al.* 1985). *Aeromonas* spp. may possess virulence factors such as proteases, enterotoxins, hemolysins, endotoxins and cytotoxins (Turnbull *et al.* 1984, Burke *et al.* 1986, Watson *et al.* 1985, Stelma *et al.* 1986, Barer *et al.* 1986). A number of virulence factors of motile aeromonads is given in table 2. The following observations have been used to argue the enteropathogenicity of the aeromonads (Van der Kooij 1988):

- aeromonads possess a series of specific properties, which are considered to be virulence factors;

- aeromonads have usually been isolated in higher frequencies from diarrhoeal stools than from healthy controls;

- in cases of diarrhoea with aeromonads, these organisms were present in large numbers than in symptomless carriers.

However, controversy still exists concerning the role of *Aeromonas* spp. in acute gastroenteritis and further research is needed (Buchanan and Palumbo 1985, Van der Kooij 1988). Species of the genus *Aeromonas* are considered to be autochthonous inhabitants of aquatic environments (Kaper *et al.* 1981), with densities depending on pollution, trophic state and temperature (Van der Kooij 1988). Motile aeromonads are mesophilic with an optimum temperature around 28E C and an usual maximum growth temperature of 38-41E C (Popoff 1984). Large numbers of aeromonads have been found in drinking water (Burke *et al.* 1984, Van der Kooij 1988, Schubert 1976), fresh water (Seidler *et al.* 1980, Rippey and Cabelli (1979), ground water (Schubert 1976), estuarine (Seidler *et al.* 1980) and saline waters (Kaper *et al.* 1981, Alonso *et al.* 1990, Arribas *et al.* 1987), and in a variety of other sources such as activated sludge (Neilson 1978), sediments (Williams and LaRock 1985) and food (Callister and Agger 1987, Palumbo *et al.* 1985). Motile aeromonads has been considered one of the most discriminating single parameter for defining trophic state in freshwater (Rippey and Cabelli 1988).

To study the ecology of *Aeromonas* spp. in water, simple but accurate and reliable isolation and identification procedures are needed. Most of the media and procedures presently employed for the isolation and enumeration of *Aeromonas* spp. are not specific and require a number of additional biochemical tests for presumptive identification and confirmation. A specific medium (ADA) developed by Havelaar *et al.* (1987), that makes use of the high specificity of dextrin fermentation for the detection of aeromonads, allowed a good recovery of *Aeromonas* spp. from different kinds of waters and was highly selective. Palumbo *et al.* (1985) described the successful development of a plating medium (SA) for the quantitative detection of *A. hydrophila*

in foods using starch hydrolysis, because this enzyme activity is mainly restricted to *Aeromonas* and *Vibrio* species. Alonso and Garay (1989) developed a membrane filtration procedure for a rapid quantitative recovery of motile *Aeromonas* from seawater in the presence of very large numbers of competing microflora. A number of media for the enumeration and isolation of motile *Aeromonas* is given in table 3.

The Mediterranean seems to produce an ideal environment for the proliferation of *Aeromonas* spp. since the climate is temperate throughout the year and the rivers have little flow and a high organic matter load, and many of the beaches affected by these rivers serve as bathing areas for thousands of people (Araujo *et al.* 1988).

Comparative studies on environmental samples have been limited. This study documents the occurrence and seasonal fluctuations of *Aeromonas* in a Mediterranean area.

Table 1

Differentiation between *Aeromonas hydrophila, A. caviae* and *A. sobria*[*]

Characteristics	*A. hydrophila*	*A. caviae*	*A. sobria*
Cell shape	Rods	Rods	Rods
Gram stain	- +	-	-
Motility	+	+	+
Cytochrome oxydase	+ +	+	+
Esculin Hydrolysis		+	-
Growth in KCN broth	+	+	-
L-histidine and L-arginine utilization	+ +	+	-
L-arabinose utilization	+	+	-
Fermentation of salicin	+	+	-
Fermentation of sucrose	-	+	+
Fermentation of manitol		+	+
Breakdown of inositol	+	-	-
Acetoin from glucose (Voges-Proskauer)	+ +	-	d
Gas from glucose		-	+
H_2S from cysteine		-	+

[*]: Symbols: +, typically positive;
-, typically negative;
d, differs among strains

Table 2

Virulence factors in *Aeromonas* species[a]
(From Van der Kooij 1988)

Virulence factor or characteristic	*A. hydrophila*	*A. sobria*	*A. caviae*	Origin of isolates[b]
Rabbit ileal loop	12/14[c]	-	-	S
	4/13	8/8	-	F
	1/7	5/7	-	E,H
	19/20	16/20	2/18	E,C
Suckling mouse	11/13	8/8	-	F
	80/103[c]	-	-	E,C
	9/21	29/31	0/12	S
Cytotoxicity				
- Y-1 adrenal cells	128/330	13/35	-	RW
- Vero cells	20/20	13/13	0/13	S
	26/26	9/22	0/13	DW
	4/9	17/21	0/39	E,C
"Asao" toxin, heat labile	15/17	13/13	-	S
	21/26	9/9	0	DW
Haemolytic activity	17/20	13/13	0/13	S
	26/26	9/22	0	D,E
	55/58	30/35	7/34	E,C
Invasiveness (HEp-2cells)	2/21	16/36	0/12	W
Mouse lethality[d]	5/13	11/11	0/8	C
Haemagglutinating activity (soluble)	40/55	70/70	51/75	S

[a]: Not all characteristics considered as virulence factors are shown
[b]: S = Stool, F = Fish, E = Environment, C = Clinical, RW = River water, DW = Drinking water
[c]: No differentiation between *Aeromonas* species based on scheme of Popoff and Veron (1976)
[d]: Intraperitoneal inoculation

Table 3

Agar media for the enumeration and isolation of *Aeromonas* in water and food

Selective substrate	Medium name	Inhibiting compound(s)	Technique[a]	Incubation temperature	Reference
Dextrin	DFS	Sodium sulfite/ fuchsin	SP/MF	35E-37E C	Schubert 1967
Starch	-	Penicillin/ pimafucin	SP/MF	25E C	Kielwein 1971
Maltose	RS	Novobiocin/Na deoxycholate	MF	37E C	Shotts 1973
Glycogen	PBS	Na laurylsulfate	PP	25E C	McCoy and Pilcher 1974
Trehalose	mA	Ampicillin/ Ethanol	MF	37E C	Rippey and Cabelli 1979
Starch	SA	Ampicillin	SP	28E C	Palumbo *et al.* 1985
Dextrin	ADA	Ampicillin	MF	30E C	Havelaar *et al.* 1987
Dextrin	ADA/ 0129	Ampicillin/ 0129	MF	30E C	Alonso and Garay 1985
Starch/ glucose	SGAP-10C	Ampicillin/ penicillin	MF/PP	28E C	Araujo *et al.* 1989
Xilose/ meso-inositol	MIX	Ampicillin	MF/anaerobic incubation	30E-35E C	Cunliffe and Adcock 1989
Pectin	PA	----	----	25E C	Myers *et al.* 1982
Trehalose	MAC	Crystal violet/ bile salts	MF	35E C	Kaper *et al.* 1981

[a]: SP = spread plate, MF = membrane filtration, PP = pour plate

1.3 *Campylobacter*

Campylobacter are slender, spirally curved rods, 0.2-0.5 µm wide and 0.5 to 5µm long. The rods may have one or more spirals and can be as long as 8 µm. They also appear S shaped and gull-winded when two cells form short chains. Cells in old cultures may form spherical or coccoid bodies. Gram negative. Motile with a characteristic corkscrew-like motion by means of a single polar flagellum at one or both ends of the cell. Microaerophilic. Require an oxygen concentration of between 3 and 15% and a carbon dioxide concentration of 3-5% (Smibert 1984).

The occurrence of campylobacters in natural water is extremely variable and it is not yet known which of the organisms isolated are pathogenic to humans (APHA 1985). Thermophilic campylobacters have received considerable attention in recent years as a major cause of bacterial enteritis in man (Jones *et al.* 1984, Fricker 1987, Skirrow 1987). *Campylobacter jejuni* can be found worldwide, and in developed countries is now recognized as one of the commonest causes of bacterial diarrhoea (Skirrow 1987). To a lesser extent, enteritis is also associated with *C. coli*. Other members of the genus occasionally may be found in surface waters but they can be distinguished from *C. jejuni* and *C. coli* by their growth and biochemical characteristics. The minimal tests to distinguish *C. jejuni* or *C. coli* from other *Campylobacter* species are indicated in table 4.

Table 4

Differential characteristics of *C. jejuni* and *C. coli* (APHA 1985)

Species	Catalase	Growth at		Growth in hippurate	Nalidixic acid sensitivity
		25E C	42E C		
C. jejuni	+	-	+	+	+
C. coli	+	-	+	-	+
C. foetus	+	+	±	-	-
C. sputorum	-	+	-	-	-

Enteritis is produced also by the Nalidixic Acid Resistant Thermophilic *Campylobacter* (NARTC) formally described as a new species with the proposed name of *C. laridis* (Benjamin *et al.* 1983), and by a fourth thermophilic species named "*C. upsaliensis*" (catalase negative or weakly reacting) (Sandstedt and Ursing 1986). The latter species has probably been largely overlooked as it is sensitive to cephalotin and other antibiotics used in *C. jejuni* isolation media (Owen and Hernandez 1990).

Thermophilic campylobacters are common gastrointestinal pathogens and they may cause more enteritis than salmonellas do (Blaser *et al.* 1983, Svedhem and Kaijser 1980). *Campylobacter* enteritis is usually a mild to moderate self-limited illness; however, patients with severe, prolonged or relapsing enteritis should be treated (Lariviere *et al.* 1986).

Improved methods for the isolation and cultivation of campylobacters have recently revealed the ubiquitous nature of these potentially pathogenic spirilla (Rollins and Colwell 1986). It is now well established that warm-blooded wild and domestic animals harbour these organisms in their intestines and thus have to be considered as important reservoirs (Rosef *et al.* 1983); avian, bovine, ovine, porcine, canine and feline sources have been identified and recognized as important reservoirs of campylobacters (Blaser *et al.* 1983, Karmali and Fleming 1979). Animal hosts of *Campylobacter* may be associated with survival of the organism in nature, since faecal material is shed directly into aqueous environment (Rollins and Colwell 1986).

Water is potentially an important reservoir of the thermophilic campylobacters and is an established vehicle for the transmission of these organisms to man and domestic animals (Hoff and Geldreich 1982, Bolton *et al.* 1987). Most human *Campylobacter* infections are contracted by ingestion of food, water or milk contaminated by the bacteria (Boosinger *et al.* 1990). There are reports of enteritis caused by waterborne, thermophilic campylobacters from many parts of the world: USA (Taylor *et al.* 1983), Sweden (Mentzing 1981), Finland (Aho *et al.* 1989), Yugoslavia (Brokovic *et al.* 1983), Norway (Gondrosen *et al.* 1985), England (Palmer *et al.* 1983, Jones *et al.* 1990a, Skirrow 1987), Israel (Rogol *et al.* 1983) and various parts of the developing world (Newell 1982). Although these water-borne outbreaks with campylobacters there have relatively few surveys to determine the distribution of these organisms in natural waters (Bolton *et al.* 1987). A number of reports describe the isolation of *Campylobacter* from various aquatic habitats, it has been found campylobacters in river water (Stelzer 1990, Kadar 1990, Fricker and Park 1989, Bolton *et al.* 1987), seawater (Jones *et al.* 1990c), sewage (Fricker and Park 1989), sewage sludge (Jones *et al.* 1990b, Lauria and Hofer 1989), ponds, lakes and small mountain streams (Carter *et al.* 1987).

It has been suggested that sewage effluent can be a source of infection for the community (Jones and Watkins 1985). Campylobacters are discharged continuously by sewage effluents into the receiving waters, recent studies have shown a high pollution of raw sewage with thermophilic campylobacters in the range of 10^2-10^4/100 ml (Stelzer *et al.* 1980). It must be remembered that the minimum infective dose for some of these organisms is very low (Robinson 1981). One epidemiological study estimated that as few as 500 *C. jejuni* organisms can initiate illness in children.

The presence of an indicator bacterium for *Campylobacter* spp. contamination would be useful to indicate the possible presence of the organism. Several studies have been done concerning the isolation of *Campylobacter* spp. from surface water in association with *Escherichia coli* (Bolton *et al.* 1982b, Knill *et al.* 1977).

The ability to recover campylobacters is influenced by technique, the physiological state of the organisms, and the number of cells present. Early investigations and development of methods for detection of *Campylobacter* spp. were largely directed toward analysis of clinical specimens. Detection of campylobacters in aqueous systems present special problems in concentration and isolation of the organism (Mathewson *et al.* 1983, Taylor *et al.* 1983). Isolation of *C. jejuni* or *C. coli* is difficult because of the competitive growth of other organisms (APHA 1985). The selective media currently used to isolate

campylobacters consists of a rich basal medium, lysed blood and various combinations of antimicrobial agents (Fricker 1987). Selective media do not eliminate all the other intestinal organisms but only limit growth of these organisms to favor isolation of campylobacters (Smibert 1984). Campylobacters are microaerophilic, requiring a low oxygen tension (3-6% oxygen) for growth, the oxygen toxicity can be overcome in solid or liquid media with very large inocula containing large numbers of cells (Smibert 1984). The use of agar media containing blood enhances the aerotolerance of campylobacters because blood contains catalase and superoxide dismutase (Smibert 1984). Various workers have used different basal media in their formulations. A number of selective media used for isolation of campylobacters is given in table 5. In comparative studies (Bolton *et al.* 1983a, Fricker *et al.* 1983) of the selective media of Skirrow, Butzler, Blaser, Campy-BAP and Preston, the Preston medium was found to give the maximum isolation rate of *Campylobacter* from all types of specimens tested and also to be the most selective. The Preston agar (Bolton and Robertson 1982a) was specifically formulated to make it suitable for isolation of *Campylobacter* species from all types of specimens (human, animal, avian and environmental). This medium is more inhibitory to competing flora than most other recommended media and this probably explains its greater sensitivity (Fricker 1987).

The inclusion of blood has several disadvantages because it is a relatively expensive commodity of variable composition and limited self-life and it is not readily available in developing countries (Bolton and Coates 1983b). It is of interest that Bolton *et al.* (1984) have developed a selective medium which does not require blood, and is similar in efficiency to other *campylobacter* selective media (Fricker 1987). Addition of 0.025% each of ferrous sulfate, sodium metabisulfite and sodium pyruvate (FBP) to culture media increase the aerotolerance of cultures of *C. foetus, C. jejuni* and *C. coli* and allow growth at oxygen concentrations of 15-20% (George *et al.* 1978, Hoffman *et al.* 1979). The FBP mixture destroys hydrogen peroxide and superoxide anions that appear in the medium when exposed to air and light (Smibert 1984).

In common with other Gram-negative bacteria, Campylobacters can suffer sub-lethal injury which sensitizes it to selective agents used in isolation media and to which undamaged cells are resistant (Humphrey and Cruickshank 1985). The methods used for isolation of campylobacters offer a means of detection and enumeration of viable but not culturable campylobacters, methods presently used for detection and enumeration must be re-evaluated and new techniques must be devised (Rollins and Colwell 1986). Sub-lethally injured cells of campylobacters from environmental samples are progressively less able to grow at 43E C, particularly on selective media. With broth culture the isolation rate could be increased by pre-enrichment in basal or selective media at 37E C for 4h (Humphrey 1989).

2. MATERIALS AND METHODS

2.1 Sampling area

The study area correspond to Valencia coastal waters (Figure 1). Samples were taken from 8 sampling sites. The distribution of each sampling site is given in: figure 2 (Puig, Puzol and Puebla de Farnals), figure 3 (Port Saplaya and Malvarrosa), figure 4 (Saler and Perello) and figure 5 (Cullera). Samples at each site were provided as follows: one near from the sewage discharge area (site 1) and second separated 200 meters from the first point (site 2).

Water samples were collected at monthly intervals during the period from January to December 1990. Samples were transported at 4E C from the sampling sites to the laboratory. The period of time between collecting the samples and initiating waters tests never exceeded 2h.

2.2 Enumeration of indicator organisms

2.2.1 Total coliforms by MPN technique

For total coliforms a series of five fermentation tubes of lauryl tryptone broth (Oxoid) were inoculated with appropriate decimal quantities (multiplies and submultiplies of 1 ml of sample), to determine the most probable number (MPN) of coliforms (APHA 1985). The tubes were inoculated at 37E C for 48h. Formation of gas in any amount in the inverted tubes within 48h was considered a positive reaction.

2.2.1.1 Lauryl tryptone broth (this medium is available commercially).
Single strength formulation.

Tryptone	20.0 g
Lactose	5.0 g
K_2HPO_4	2.75 g
KH_2PO_4	2.75 g
NaCl	5.0 g
Sodium lauryl sulfate	0.1 g
Distilled water	1000.0 ml

The addition of a pH indicator (bromocresol purple, phenol red) to presumptive medium is optional as a guide to determine acid productivity and growth (without gas formation) to be confirmed.

The pH of the medium should be 6.8 ± 0.2 after sterilization. Before sterilization, the medium was dispensed in fermentation tubes with an inverted vial. The medium was autoclaved at 121E C for 15 min. A double strength of lauryl tryptone broth was prepared for a volume of 10 ml of sample.

2.2.1.2 Dilution solutions: phosphate buffer (pH 7.2) for dilutions.

K_2HPO_4	3.0 g
KH_2PO_4	1.0 g
Distilled water	1000.0 ml

Table 5

Selective media used for isolation of campylobacters

Medium name	Agar base	Blood	Selective supplement	Reference
Skirrow	Blood agar	5 to 7% lysed horse blood	Vancomycin (10 mg/l) Polymyxin (2500 U/l) Trimethoprim (5 mg/l)	Skirrow 1977
Butzler	Thyoglycollate agar	15% defibrinated whole sheep blood	Bacitracin (2500 U/l) Cycloheximide (50 mg/l) Colistin sulphate (10000 U/l) Cefazolin (15 mg/l) Novobiocin (5 mg/l)	Butzler and Skirrow 1979
Campy-BAP	Brucella medium base	10% defibrinated whole sheep blood	Vancomycin (10 mg/l) Trimethoprim (5 mg/l) Polymixin B (2500 U/l) Cephalotin (15 mg/l) Amphotericin B (2mg/l)	Blaser *et al.* 1979
Preston	Nutrient broth No. 2 and agar	5% lysed horse blood	Polymixin B (5000 U/l) Rifampicin (10 mg/l) Trimethoprim (10 mg/l) Cycloheximide (100 mg/l)	Bolton and Robertson 1982a
CCDA	Nutrient broth No. 2 Casein hydro-lysate Sodium deoxycholate Ferrous sulphate Sodium pyruvate		Cefoperazone (32 mg/l)	Bolton *et al.* 1984

N 4

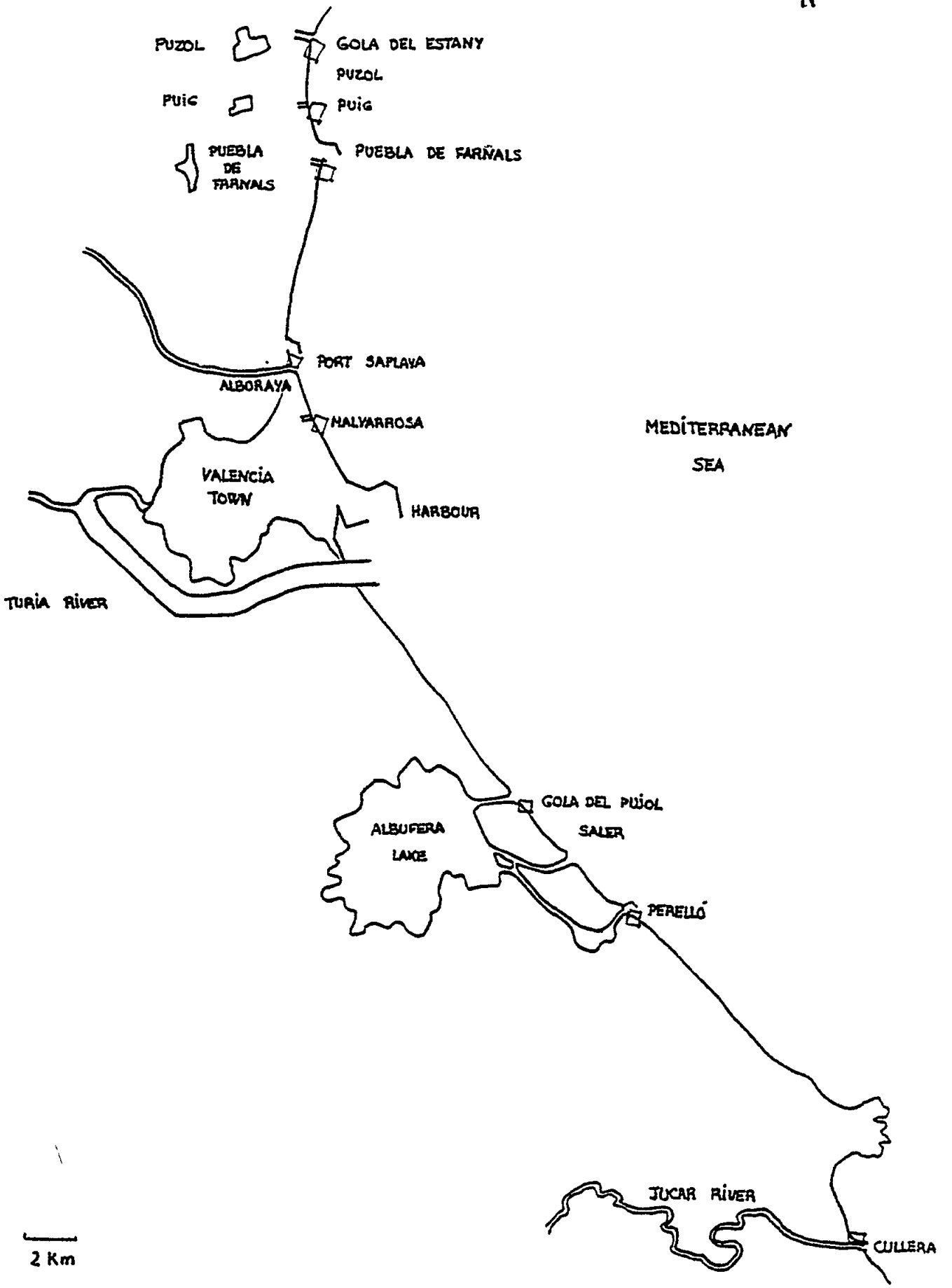

Figure 1 - Geographical distribution of sampling areas

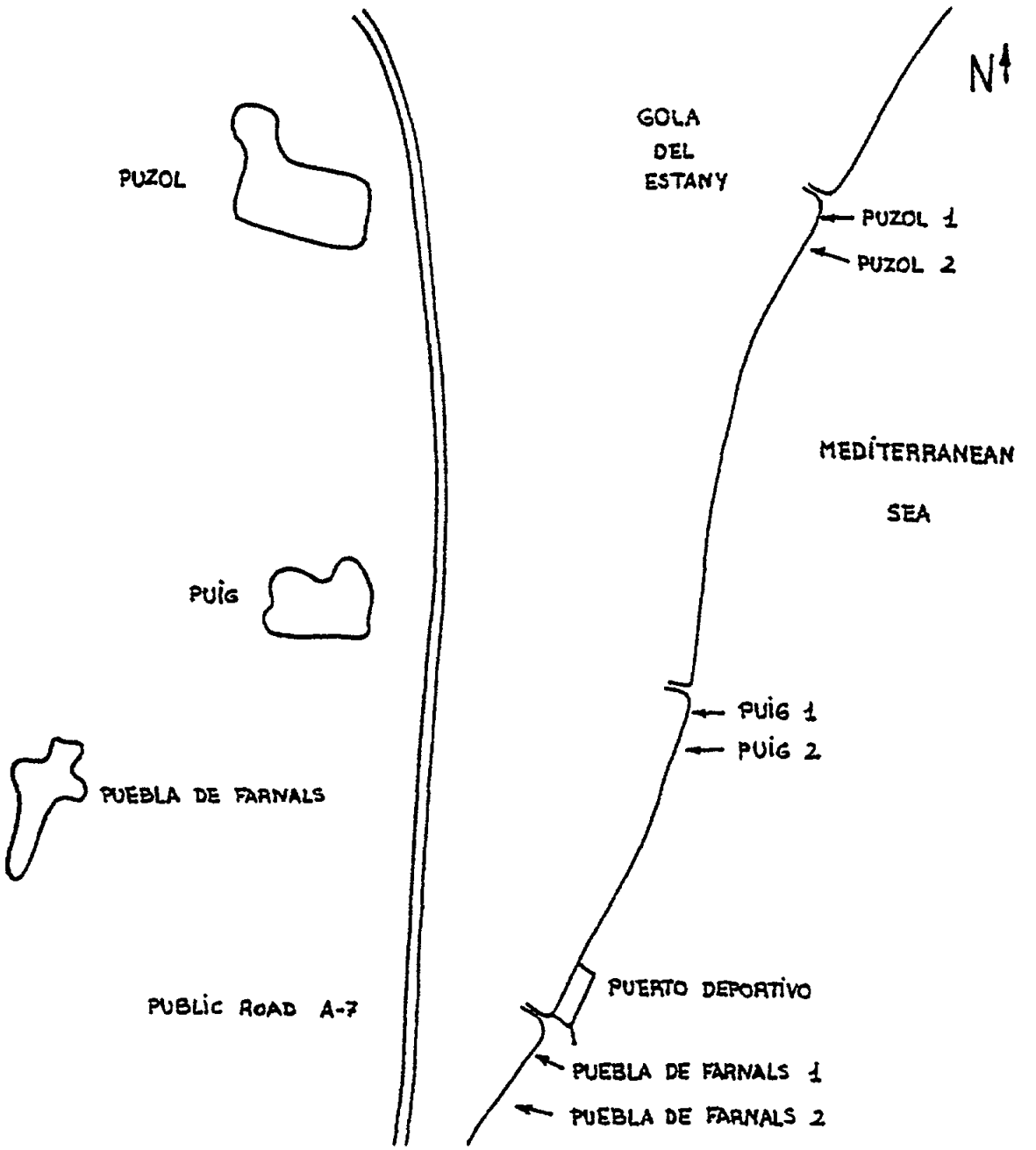

Figure 2 - Geographical distribution of Puzol (sites 1 and 2), Puig (sites 1 and 2) and Puebla de Farnals (sites 1 and 2)

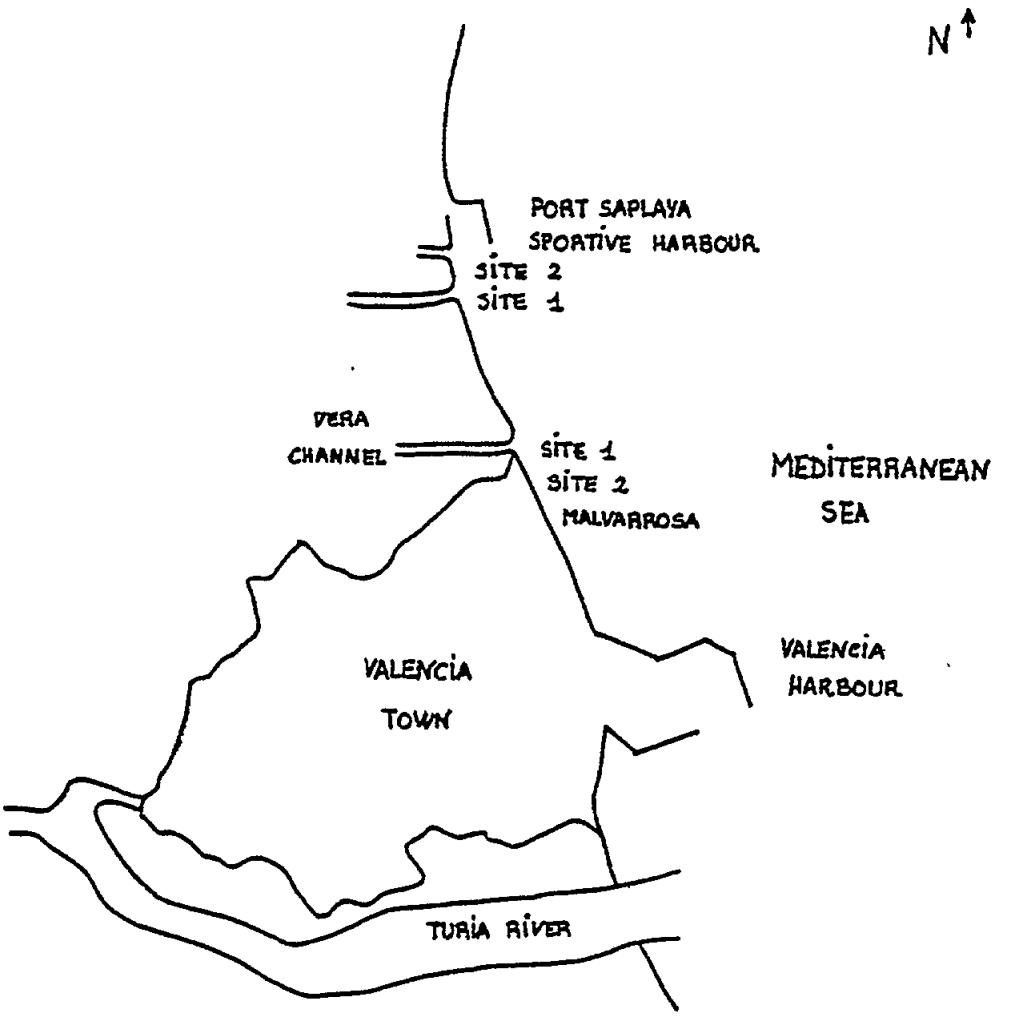

Figure 3 - Geographical distribution of Port Saplaya (sites 1 and 2) and Malvarrosa (sites 1 and 2) sampling areas

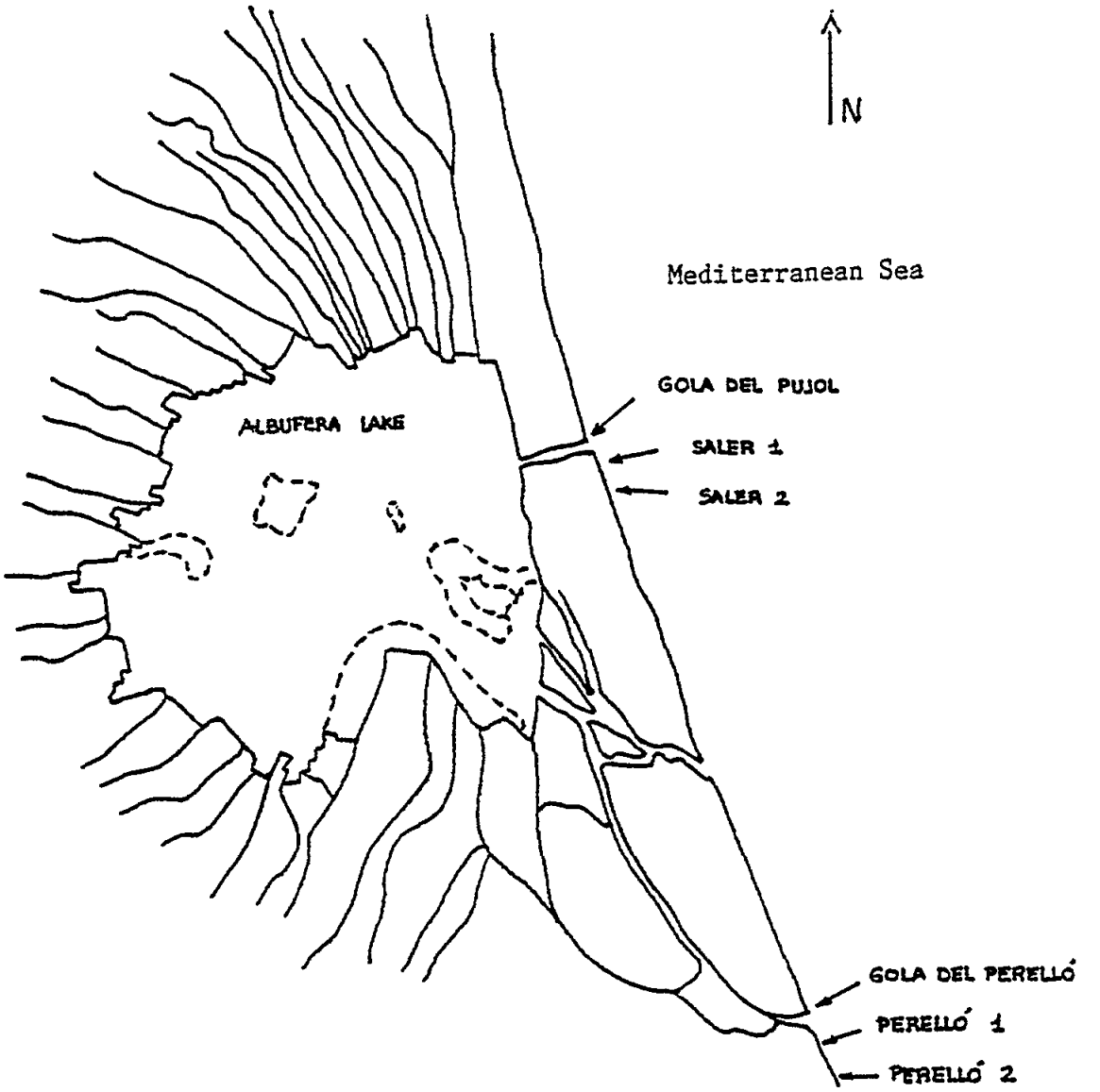

Figure 4 - Geographical distribution of Saler (sites 1 and 2) and Perello (sites 1 and 2) sampling areas

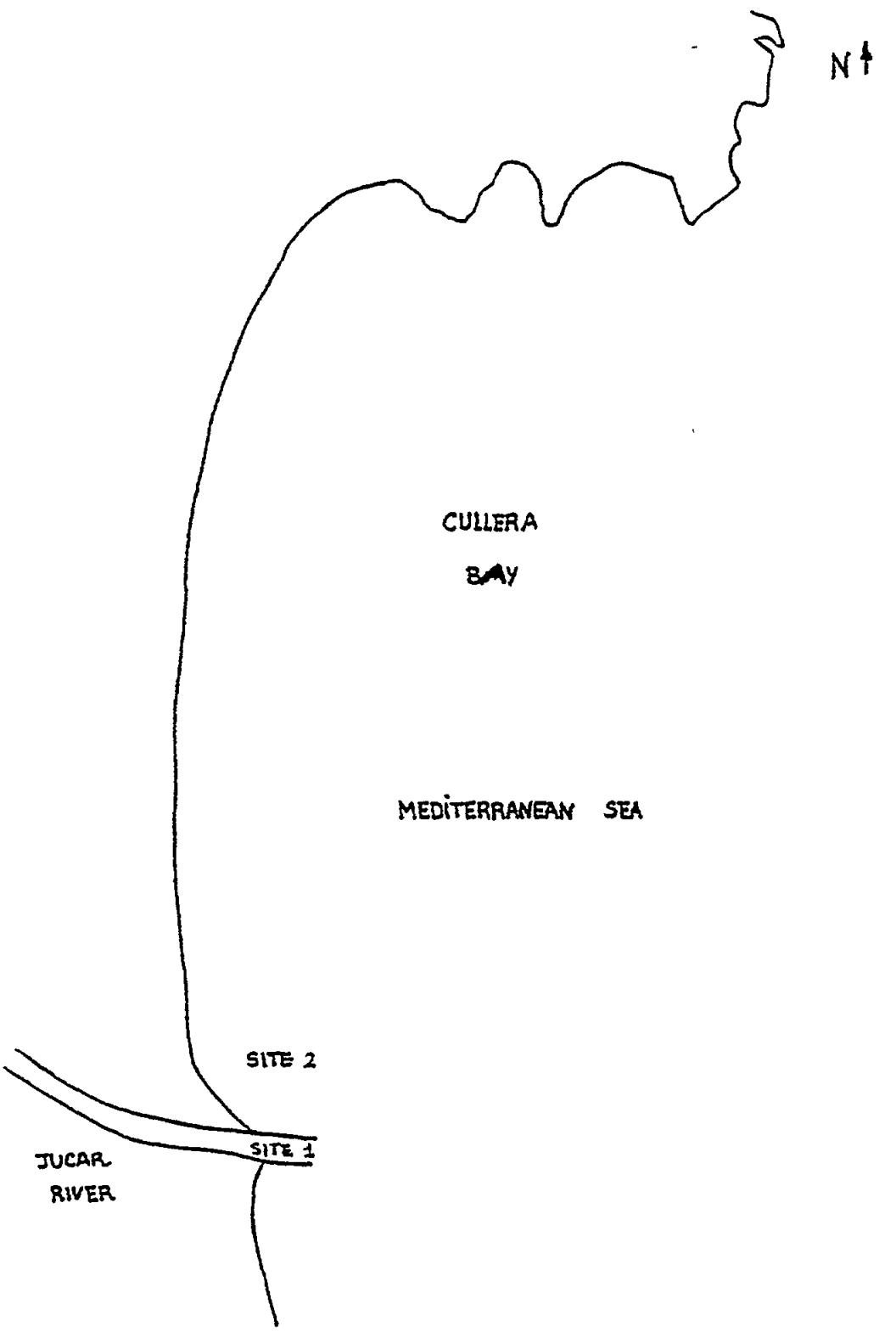

Figure 5 - Geographical distribution of sites 1 and 2 at Cullera bay

The ingredients were dissolved and dispensed 9 ml in test tubes used for dilutions in the dilution series and autoclaved at 121E C for 15 min.

2.2.2 Faecal coliforms by MPN technique

All presumptive tubes which were positive as defined in Standard Methods (APHA 1985) were transferred (one drop) into EC broth (Difco) and incubated for 24h at 44.5 ± 0.2E C to determine faecal coliforms. Gas production in a fermentation tube within 24h or less was a positive reaction indicating coliforms of faecal origin.

2.2.2.1 EC medium: (this medium is available commercially).

Tryptone	20.0 g
Lactose	5.0 g
Bile Salts No. 3	1.5 g
K_2HPO_4	4.0 g
KH_2PO_4	1.5 g
NaCl	5.0 g
Distilled water	1000.0 ml

The pH of the medium should be 6.9 +0.2 after sterilization. Before sterilization, the medium was dispensed in fermentation tubes, each one with an inverted vial. The medium was autoclaved at 121E C for 15 min.

2.2.3 Resuscitation procedure of faecal coliforms

Faecal coliforms were enumerated by membrane filtration, using m-T7 agar (LeChevallier *et al.* 1983). This medium was designed to improve recoveries of injured faecal coliforms. Bromothymol blue and bromocresol purple were added as indicators of lactose fermentation. The tergitol-7 and polyoxyethylene ether W-1 were incorporated as selective agents. Additional selectivity may be obtained by aseptically adding penicillin G (0.1 µg/ml) to the medium after autoclaving.

After filtration of water samples, the filters (HAWG 097, Millipore Corp. Bedford Mass.) were placed on the plates, which were preincubated at 37E C for 8h before transfer to 44.5E C for 12h. With this preincubation time, significant increases in isolation rate of faecal coliforms were described (LeChevallier *et al.* 1984).

2.2.3.1 m-T7 agar: (this medium is available commercially)

Proteose peptone No.3	5.0 g
Lactose	20.0 g
Yeast extract	3.0 g
Tergitol 7 (25% solution)	0.4 ml
Polyoxyethylene ether W-1	5.0 g
Bromothymol blue	0.1 g
Agar	15.0 g
Distilled water	1000.0 ml

The final pH of the medium should be 7.4. The medium was autoclaved at 121E C for 15 min.

2.2.3.2 Penicillin stock solution 0.01 mg: the penicillin G solution was filter sterilized.

The plates of m-T7 agar may be stored at refrigerator temperature for up to 1 week provided excessive drying does not occur.

All smooth, yellow, convex colonies on m-T7 agar were counted as faecal coliforms.

Confirmation of isolates: a number of 366 typical colonies from m-T7 agar were verified by inoculation into lauryl tryptone broth (37E C for 48h) followed by transfer to EC broth (44.5E C for 24h).

2.3 Motile *aeromonas* enumeration

Motile aeromonads were enumerated by membrane filtration, using mADA/0129 agar (Alonso and Garay 1989). The medium was prepared according to the instructions of Havelaar and Vonk (1988), adding the vibriostatic agent 0/129 (Sigma Chemical Co.) at 50 mg/l as recommended by Havelaar *et al.* (1987) for seawater samples. Previous experiments in our laboratory using ATB Gram$^-$ system (API lab.) with aeromonads environmental strains, have revealed that they grew perfectly at the highest concentration of ampicillin (16 mg/l) (Amoros *et al.* 1989). So we decided to increase the antibiotic concentration up to that value in mADA agar. After filtration of water samples, the filters HAWG 047 (Millipore Corp.) were placed on the plates, which were incubated 24h at 30E C. Those colonies showing dextrin fermentation were scored as presumptive motile aeromonads.

2.3.1 Confirmation of isolates

Presumptive aeromonads strains isolated from mADA/0129 agar were confirmed by using the following protocols, tests and media: oxidase, growth on TSI agar (Difco) and *A. hydrophila* confirmation medium (Kaper *et al.* 1979). Strains that showed the following biochemical features in AH medium: manitol (+), decarboxylation ornitine (-), H_2S (-), motility (+) and indole (+) were scored as motile aeromonads and further tested for acid and gas production from glucose, and esculine hydrolysis. The characteristics of the isolates were compared against those described by Popoff (1984). The specificity of mADA/0129 agar was determined from biochemical tests performed on presumptive positive colonies isolated from the medium.

The incubation temperature used during this study was 30E C and the readings of all the identification tests were performed at 48h.

2.3.2 Preparation of media and solutions

2.3.2.1 mADA/0129 agar: (this medium is not available commercially).

Basal medium:

Tryptone	5.0 g
Yeast extract	2.0 g
Dextrin weiss (Merck)	10.0 g
NaCl	3.0 g

KCl	2.0 g
$MgSO_4.7H_2O$	0.2 g
$FeCl.6H_2O$	0.1 g
Bromothymolblue solution	8.0 ml
Agar	15.0 g
Distilled water	962.0 ml

Bromothymolblue solution: 1.0 g of bromothymolblue was dissolved in NaOH (5 mol/l) and distilled water added to 100 ml.

After dissolving these components the pH was adjusted to 8.0 with NaOH (5 ml/l) and agar was added and dissolved by gentle boiling. The medium was autoclaved for 15 min at 121E C. Basal medium may be stored 1 month at 4-6E C until use.

Solutions supplement:

Ampicillin	10 ml
Sodium deoxycholate	10 ml
0/129	10 ml

1. Sodium ampicillin solution: 16 mg of sodium ampicillin (Sigma Chemical Co.) was dissolved in 10 ml of distilled water and sterilized by filtration.

2. Sodium deoxycholate solution: 100 mg of sodium deoxycholate (Difco) was dissolved in 10 ml of distilled water and sterilized by filtration.

3. Vibriostatic agent 0/129 solution: 50 mg of 0/129 (Sigma Chemical Co.) was dissolved in 10 ml 50/50 acetone/distilled water and sterilized by filtration. After autoclaving the basal medium and tempering to 45-50E C, the sodium ampicillin, sodium deoxycholate and 0/129 solutions were added successively. Plates were poured and kept in a refrigerator for not longer than a week.

2.3.2.2 Oxidase test (Yu and Washington 1985): (this test is available commercially).

Cytochrome oxidase is a heme-containing protein component in the respiratory chain of enzymes responsible for reactions taking place during oxidative phosporilation. Cytochromes are found in bacteria that can use oxygen as a final electron acceptor in their energy metabolism and are therefore absent in strict anaerobes.

In the oxidative test, a substrate, 1% tetramethyl-p-phenylene diamine dihydrochloride, is oxidized to a purple colored substance. Only fresh reagent should be used, because it is readily oxidized on standing in air. Also, a platinum inoculating loop, rather than a nichrome loop or loop containing an oxidizing substance, should be used to prevent false positive results.

Motile Aeromonas analysis:

- Enumeration: Sample
  Filter measured volume
  ↓
  agar mADA/0129 (-yellow or yellow-orange colonies= presumptive Aeromonas spp.
  30ºC for 24h
  (photo 10) -blue colonies or microcolonies= non-Aeromonads

- Identification: Purification on Nutrient agar
  ↓
  Oxidase test
  ↓
  Growth on TSI agar: lactose +, glucose +, gas + or -, sulphide production -
  ↓
  Growth on AH medium: manitol +
  (photo 11) inositol +
  ornitine decarboxilation -
  H_2S -
  Motility +

- Species identification: glucose broth

(photo 12) esculine agar

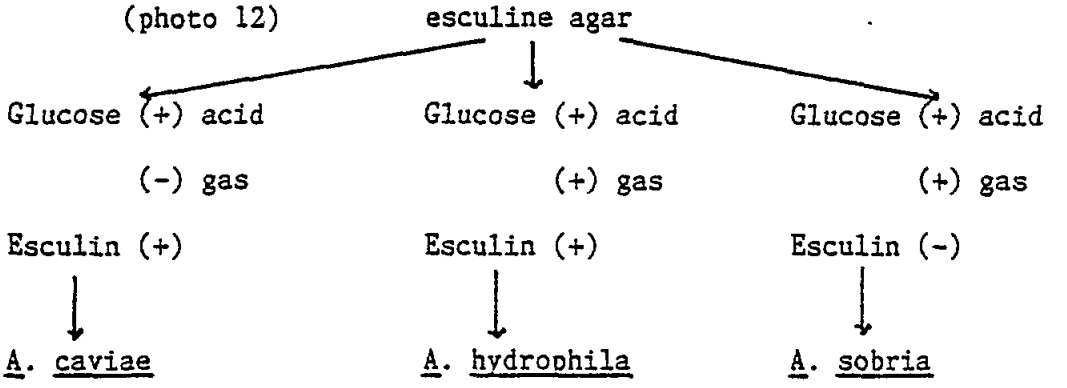

Figure 6 - Schematic diagram for isolation, enumeration and identification of motile *Aeromonas*

Oxidase reagent:

N,N,N', N'-tetramethyl-phenylenediamine dihydrochloride 1 g
Distilled water in 100 ml dissolved by heating if necessary.

A piece of filter paper was placed in a petri dish and saturate it with oxidase reagent. With a platinum loop, a portion of a colony was picked and rubbed gently onto the impregnated filter paper. A positive test was considered when a colony deposit turns purple within seconds.

2.3.2.3 Triple sugar Iron Agar: (this medium is available commercially).

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Sodium chloride	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose	1.0 g
Ferric citrate	0.3 g
Sodium thiosulphate	0.3 g
Phenol red	0.024 g
Agar	12.0 g
Distilled water	1000.0 ml

After dissolving the components, the pH was adjusted to 7.4. The medium was autoclaved at 121E C for 15 min and allowed slopes to set with a generous butt.

Typical reactions in TSI agar are given in table 6.

2.3.2.4 *A. hydrophila* confirmation medium (AH) (this medium is not available commercially).

The reactions of AH medium are based upon the principles of the triple sugar iron agar, lysine iron agar, and motility-indole-ornithine medium (Kaper *et al.* 1979).

Proteose peptone	5.0 g
Yeast extract	3.0 g
Tryptone	10.0 g
L-ornithine hydrochloride	5.0 g
Manitol	1.0 g
Inositol	10.0 g
Sodium thiosulfate	0.4 g
Ferric ammonium citrate	0.5 g
Bromocresol purple	0.02 g
Agar	3.0 g
Distilled water	1000.0 ml

Table 6

Typical reactions in triple sugar iron agar tubes

Organism	Butt	Slope	H_2S
Aeromonas spp.	A or AG	A	-
Escherichia coli	AG	A	-
Proteus vulgaris	AG	A	-
Shigella sonnei	A	NC or ALK	-
Proteus morganii	AG	NC or ALK	+
Salmonella enteriditis	AG	NC or ALK	+
Salmonella choleraesuis	AG	NC or ALK	-
Pseudomonas aeruginosa	ALK	ALK	-

AG = acid (yellow) and gas formation
A = acid (yellow)
NC = no change
ALK = alkaline (red)
+ = hydrogen sulphide (black)
- = no hydrogen sulphide (no black)

The ingredients were mixed in 1 liter of distilled water and the pH was adjusted to 6.7. The medium was heated to a boil, dispensed in 5-ml quantities in tubes (13 by 100 mm), and autoclaved at 121E C for 12 min. Colonies of *Aeromonas* were picked directly from isolation plates or membrane filters and inoculated into the medium by stabbing to the base of the tube with a straight needle. The inoculated tubes were incubated at 35E C for 18 to 24h, after which reactions were recorded. For the detection of indole production, 3 to 4 drops of Kovacs reagent were added to each tube.

In table 7 are listed the reactions in AH medium of motile aeromonads and enteric bacteria.

Kovac's reagent: (this reagent is available commercially).

Amyl or isoamyl alcohol	150 ml
p-Dimethylaminobenzaldehyde	10 g
Concentrated HCl	50 ml

Aldehyde was dissolved in the alcohol and then added the acid. The solution should have a light yellow color. If a brown color results, the reagent should not be used. Kovac's reagent is stable at 4E C for 1 year.

Table 7

Reactions of Enteric bacteria in AH medium

Species	Reaction[a]				
	Top	Butt	Motility	H_2S	Indole
Aeromonas spp.	K	A	+	-	+
K. pneumoniae	A	A	-	-	-
K. oxytoca	A	A	-	-	+
E. coli	K	K or A	+ or -	-	+
Salmonella spp.	K or A	K or A	+	+	-
Enterobacter spp.	K or N	K or N	+	-	-
Proteus spp.	R	K or A	+	+ or -	+
Y. enterocolitica	K or N	K or N	-	-	+ or -
Citrobacter spp.	K	A or K	+	+	-
Serratia spp.	N or K	N or K	+	-	-

[a] Symbols: K, alkaline reaction; A, acid reaction; R, red;
N, bleached neutral color due to the destruction of indicator;
+, 90% or more positive; -, 90% or more negative

2.3.2.5 Acid and gas formation from glucose

This test was performed in tryptone water with a glucose solution.

Tryptone	5.0 g
Yeast extract	10.0 g
Indicator solution	2.0 ml
Distilled water	1000.0 ml

Indicator solution:

Bromothymol blue	8.0 g
95% ethyl alcohol	250.0 ml
Distilled water	250.0 ml

Indicator is dissolved first in alcohol and then water is added.

The medium was dispensed in tubes with an inverted vial and autoclaved at 121E C for 15 min. When tubes are cooled, aseptically was added 0.5 ml of glucose solution to give a final concentration of 1%.

Glucose solution:

Glucose	10.0 g
Distilled water	100.0 ml

Glucose solution was filter sterilized.

2.3.2.6 Esculin Agar (Yu 1985): (this medium may be not available commercially).

Esculin is hydrolized to glucose and esculectin. The latter combines with ferric ion in the medium to form a black complex.

Esculin	1.0 g
Ferric citrate	0.5 g
Heart infusion agar	40.0 g
Distilled water	1000.0 ml

After dissolving the components the pH was adjusted to 7.0. The medium was autoclaved at 121E C for 15 min and allowed agar to harden in slant position.

2.3.2.7 Nutrient agar: (this medium is available commercially).

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Distilled water	1000.0 ml

The ingredients were dissolved by boiling. The medium was autoclaved at 121E C for 15 min and allowed agar to harden in slant position. Final pH should be 6.8. The medium may be stored 4 weeks at 4E C.

2.4 *Campylobacter* presence

Methods for isolating campylobacters are not standardized and must be considered research procedures subject to modification (APHA 1985).

2.4.1 Concentration technique

For samples near sewage discharge point (sites 1) 100 ml of water were filtered through 0.45 µ sterile membranes (Millipore Corp., Bedford Mass.) and for samples from sites 2 (separated 200 meters from site 1) 1000 ml of water were filtered through 0.45 µ sterile membrane.

2.4.2 Enrichment

After filtration, the 0.45 µ membranes were removed and introduced in flasks with 100 ml of Preston *Campylobacter* Selective Enrichment Broth. The flasks were placed in an anaerobic jar (in an atmosphere containing 5% O_2 and 10% CO_2) and preincubated at 37E C for 4h (to recover damage cells) before transfer to 42E C for 44h.

Preston selective enrichment broth: (this medium is available commercially.

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Distilled water	950.0 ml

The pH of the medium should be 7.5±0.2. The medium was autoclaved at 121E C for 15 min. After sterilization, the medium was cooled at 50E C and added aseptically 50 ml of sheep blood, 1 vial of Preston *Campylobacter* selective supplement and 1 vial of *Campylobacter* growth supplement.

Selective supplement: (this reagent is available commercially) 1 vial contents to supplement 500 ml of medium:

Polymixin B	2,500.0 U
Rifampicin	5.0 mg
Trimethoprim lactate	5.0 mg
Cycloheximide	50.0 mg

The selective supplement was reconstituted with 2 ml of 50/50 Acetone/sterile distilled water.

Campylobacter growth supplement: (this reagent is available commercially) 1 vial contents to supplement 500 ml of medium:

Sodium pyruvate	0.125 g
Sodium metabisulphite	0.125 g
Ferrous sulphate	0.125 g

The growth supplement was reconstituted with 2 ml of sterile distilled water.

2.4.3 Selective growth

From each enrichment flask a loop was streaked on plates (two per flask) of Preston *campylobacter* selective agar, which were placed in an anaerobic jar containing 5% O_2 and 10% CO_2, and incubated for 48h.

Preston *campylobacter* selective agar: (this medium is available commercially).

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Distilled water	950.0 ml

The pH of the medium should be 7.5±0.2. The medium was autoclaved at 121E C for 15 min. After sterilization the medium was cooled at 50E C, and added aseptically 50 ml of sheep blood, 1 vial of Preston *Campylobacter* selective supplement and 1 vial of *Campylobacter* growth supplement. All the ingredients were well mixed and poured into sterile petri dishes.

2.4.4 Confirmation of colonies

Confirmation of the growth of campylobacters was made from colony morphology and microscopic appearance (gram staining and acridine fluorescence staining) (campylobacters are characteristically Gram negative and curved on spiral in shape).

Cultures of campylobacters were purified by repeated subculturing on blood agar and Preston agar and tested for: catalase, nalidixic acid sensitivity (30 µg disk) and growth in hippurate. These tests are minimal to distinguish *C. jejuni* or *C. coli* from other *Campylobacter* species.

Maintenance of cultures: the *Campylobacter* cultures were maintained in 10% glycerol nutrient broth at -20E C for long-term storage.

Maintenance medium: (this medium is not available commercially).

Nutrient broth No. 2	90.0 ml
Glycerol	10.0 ml

In a 2 ml tube were added 0.5 ml of maintenance medium and 20-30 glass beads. The medium with glass beads was sterilized at 121E C for 15 min.

2.4.4.1 Microscopic examination of campylobacters

Gram staining modified technique (Merino *et al.* 1986): the counterstain safranin was used after smear decolorization with acetone-alcohol and washing with distilled water. The smear was covered with safranine for 5 min.

Also it is possible use the basic fuchsin technique (10 g/l of basic fuchsin for 10-20 seconds) of Park *et al.* (1983) as a rapid diagnosis method for staining campylobacters.

Acridine orange fluorescence technique (Cheesbrough 1984): acridine orange is a fluorochrome that causes deoxyribonucleic acid (DNA) to fluoresce green and ribonucleic acid (RNA) to fluoresce orange-red.

This technique can be used for the rapid detection of campylobacters. The dried smear was covered with the acridine orange acid stain for 5-10 seconds (the acid fixative is contained in the stain). The excess of stain was washed, and the smear decolorized with alcohol saline solution for 5-10 seconds. The smear was rinsed with physiological saline and placed the slide in a draining track. A drop of saline or distilled water was added to the smear, and covered with a cover glass. The smear was examined by fluorescence microscopy using a BG 12 exciter filter and No. 44 and No. 53 barrier filters. The smear was first examined with the 10X objective to see the distribution of fluorescing material, and then with the 40X objective and 100X objective with immersion oil (low fluorescence).

2.4.4.2 Acridine orange acid stain: (this reagent is not available commercially).

Acridine orange	0.26 g
Acetic acid, glacial	20.0 ml
Distilled water	980.0 ml

The stain is stable for several weeks.

2.4.4.3 Alcohol saline solution: (this reagent is not available commercially).

Ethanol or methanol absolute	20.0 ml
Sodium chloride, (8.5 g/l)	980.0 ml

The reagent is stable for several months.

2.4.4.4 Sodium chloride, 8.5 g/l (0.85% w/v): (this reagent is available commercially).

Sodium chloride	8.5 g
Distilled water	1000.0 ml

The reagent is stable for several months.

Catalase test (Yu and Washington 1985): the enzyme catalase decompose hydrogen peroxide to water and oxygen. Catalase is an hemoprotein. The reaction is simply represented by the following equation:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

One portion of an isolated colony was transferred from a blood-free medium, since red cells possess catalase activity, to a tube with 3% hydrogen peroxide reagent. The evolution of gas bubbles was considered a positive test.

Reagent 3% hydrogen peroxide:

Stock solution, 30% H_2O_2	10 ml
Distilled water	90 ml

The reagent may be stored in brown bottle for 6 months.

Sensitivity to nalidixic acid (30 µg) disk (Yu and Washington (1985)

A light suspension of campylobacters was streaked over the surface of sheep blood agar, and a nalidixic acid (30 µg) disk was applied. The plate was incubated at 37E C for 1-2 days in microaerophilic atmosphere. A zone of inhibition of 20 mm diameter was considered positive.

Sheep blood agar: (the basal medium and sheep blood are available commercially).

Nutrient broth No. 2	25.0 g
Agar	15.0 g
Distilled water	1000.0 ml
Sheep blood	50.0 ml

The base medium was sterilized at 121E C for 15 min. After the sterilization the medium was transferred to a 50E C water bath. When the agar was cooled to 50E C, the sterile blood was added aseptically and mixed gently but well. The blood must be allowed to warm at room temperature before being added to the molten agar. 15 ml amounts of medium

were dispensed in sterile petri dishes. The plates may be stored at 2-8E C in sealed plastic bags for 4 weeks.

Hippurate hydrolysis (Yu and Washington 1985): the hydrolysis of sodium hippurate by hippuricase to sodium benzoate and glycine is represented by the following equation:

Sodium hippurate hippuricase sodium benzoate + glycine

Subsequent addition of ninhydrin results in the oxidative deamination of the á-amino group in glycine to its corresponding aldehyde, with the release of carbon dioxide, ammonia, and hydrindantin, the reduced form of ninhydrin. Ammonia reacts with residual ninhydrin and hydrindantin to give a purple-colored complex.

The hippurate broth was inoculated heavily with 18 to 24h old colonies to produce a milky suspension. The suspension was incubated for 2h at 35E C. After this time, 0.2 ml of ninhydrin reagent was added, mixed well and incubated for additional 10 to 15 min. Positive hippurate hydrolysis was indicated by the presence of a deep purple color. A light inoculum or use of an old culture may give a gray to very slight purple color; this should be interpreted as a negative result.

Hippurate broth:

Sodium hippurate	1.0 g
Distilled water	1000.0 ml

0.4 ml of hippurate broth was dispensed in small screw-capped vials. The reagent may be stored for 6 months.

Ninhydrin reagent:

Ninhydrin	3.5 g
Acetone-butanol mixture, 1:1	100.0 ml

The reagent may be stored at room temperature for 12 months.

2.5 Statistical analysis

The bacteriological counts were logarithmically transformed. Bravais-Pearson correlation coefficients between faecal indicators and motile aeromonads were calculated with the Microstat (Ecosoft Inc.) statistical data analysis program. Statistical comparisons were made by analysis of variance and were calculated also with the Microstat program. The effect of enumeration technique was analyzed.

3. RESULTS

3.1 Total coliforms and faecal coliforms by MPN technique

The results obtained at each sampling site area were:

Puzol: total coliforms (table 8) values remained low all the year at sites 1 and 2, except the sampling of 14 May at site 1 where an increase in total coliform count was detected. Faecal coliforms levels were also low at both sites (table 16), except the samplings of 5 March, 14 May and 17 December at site 1, and 17 December at site 2. A good correlation (significance level 1%) between total coliforms and faecal coliforms was found at both sites (table 24).

Puig: the results of total coliforms and faecal coliforms at sites 1 and 2 are indicated in tables 9 and 17, respectively. Site 1 showed the highest levels of faecal pollution with maximum values above 10^5/100 ml for total coliforms and 10^4/100 ml for faecal coliforms in all samplings, except the sampling of 5 March. At site 2, total coliforms and faecal coliforms remained high, with levels of total coliforms ranging from $11x10^5$/100 ml to $79x10^2$/100 ml and faecal coliforms from $13x10^4$/100 ml to $11x10^2$/100 ml. Correlation coefficients obtained between total coliforms and faecal coliforms at both sites were very high (significance level 1%) (table 24).

Puebla de Farnals: the densities of total coliforms (table 10) and faecal coliforms (table 18) were high in the samples of site 1, illustrating the presence of a contaminating discharge of faecal origin. The concentrations of total coliforms fluctuated from $17x10^4$/100 ml to $79x10^{10}$/100 ml and faecal coliforms were found in the range of $70x10^3$/100 ml to $35x10^7$/100 ml. Site 2 presented lower levels of both indicators than site 1, the results demonstrated a high variation in the levels of total coliforms ($14x10^6$/100 ml to 80/100 ml) and faecal coliforms ($17x10^4$/100 ml to 13/100 ml). Total coliforms and faecal coliforms showed a strong correlation coefficient (significance level 1%) at both sites (table 24).

Port Saplaya: the counts of total coliforms (table 11) and faecal coliforms (table 19) were considerably high at site 1, with values above 10^5/100 ml for total coliforms and 10^3/100 ml for faecal coliforms. Site 2 showed low levels of total coliforms (table 11) and faecal coliforms (table 19) from January to May, however the bacterial indicators levels increased considerably from June to December, this was due to the presence of a new sewage flow at this site. The correlation between total coliforms and faecal coliforms was good (significance level 1%) at both sites.

Malvarrosa: the levels of total coliforms (table 12) and faecal coliforms (table 20) were similar at site 1 and 2, with low variations through the year. The highest count of total coliforms at both sites was $23x10^4$/100 ml. The numbers of faecal coliforms were, in general, above $20x10^2$/100 ml at both sites. A significant correlation at 1% level was observed between total coliforms and faecal coliforms (table 24) at both sites.

Saler: total coliforms (table 13) and faecal coliforms (table 21) exhibited, in general, similar fluctuations throughout the sampling period. This is supported by the fact that a high correlation coefficient (significance level 1%) between total coliforms and faecal coliforms was obtained at sites 1 and 2 (table 24). Bacterial indicators levels at both sites not exhibited seasonal fluctuations, and was independent of environmental temperatures. The highest

levels of total coliforms and faecal coliforms at site 1 were $35x10^3$/100 ml and $17x10^3$/100 ml respectively. At site 2 the counts of total coliforms and faecal coliforms were lower than site 1. Correlation coefficient between total coliforms and faecal coliforms was high (significance level 1%) at both sites (table 24).

Perello: the highest counts of total coliforms (table 24) and faecal coliforms (table 22) were obtained during the summer period at both sites. The site 2 is less influenced by the sewage pollution of Albufera lake, because is far (200 meters) from the drain of faecal contaminated water. Levels of total coliforms and faecal coliforms at site 1 ranging from $50x10^5$/100 ml to $23x10^2$/100 ml and $11x10^5$/100 ml to 540/100 ml respectively. The total coliforms and faecal coliforms were found to be strongly correlated (significance level 1%) at both sites (table 24).

Cullera: the highest recorded counts of total coliforms (table 15) and faecal coliforms (table 23) for the year were at site 1, approximately 100 times greater than site 2. The greatest count of total coliforms at site 1 was $14x10^4$/100 ml and $50x10^3$/100 ml respectively. The low levels of total coliforms at site 2 were also reflected in the low levels of faecal coliforms. Significant correlation (significance level 1%) was observed for total coliforms and faecal coliforms at both sites (table 24).

3.2 Bacterial water quality classification

The EEC Directive (Council Directive 76/160/EEC) and the Spanish law (Real Decreto 734/1988) for bathing waters specify mandatory `I' and guide `G' values for bacterial indicators, being respectively 10,000 and 500/100 ml for total coliforms and 2,000 and 100 for faecal coliforms. It is specified that the `I' values must not be exceeded by 95% of samples and the `G' values by 80%. The sampling sites of Puzol (site 1), Puig (sites 1 and 2), Puebla de Farnals (sites 1 and 2), Port Saplaya (sites 1 and 2), Malvarrosa (sites 1 and 2), Saler (sites 1 and 2), Perello (sites 1 and 2) and Cullera (site 1) failed the EEC imperative (I) for total coliforms and faecal coliforms (table 25).

Table 8

Counts of total coliforms by MPN technique at Puzol sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
15 January	278	221
5 February	3,300	1,600
5 March	8,000	2,300
17 April	22	23
14 May	13,000	1,600
11 June	300	80
9 July	1,700	900
23 July	300	170
17 September	4,900	920
15 October	1,700	540
12 November	7,900	350
17 December	7,000	4,900

Table 9

Counts of total coliforms by MPN technique at Puig sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
15 January	180,000	49,000
5 February	140,000	11,000
5 March	8,000	8,000
17 April	1,300,000	50,000
14 May	13,000,000	140,000
11 June	700,000	300,000
9 July	22,000,000	13,000
23 July	14,000,000	80,000
17 September	4,900,000	1,100,000
15 October	230,000	33,000
12 November	7,900,000	3,300,000
17 December	330,000	7,900

Table 10

Counts of total coliforms by MPN technique at Puebla de Farnals sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
15 January	2,780,000	18,000
5 February	490,000	4,900
5 March	2,800,000	5,000
17 April	170,000	80
14 May	2,200,000	13,000
11 June	23,000,000	80,000
9 July	1,300,000,000	3,000
23 July	1,700,000,000	170,000
17 September	1,400,000,000	1,400,000
15 October	22,000,000	28,000
12 November	790,000,000,000	14,000,000
17 December	170,000,000	33,000

Table 11

Counts of total coliforms by MPN technique at Port Saplaya sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
15 January	27,800,000	13,000
5 February	490,000	23,000
5 March	220,000	80,000
17 April	280,000	2,200
14 May	1,300,000	9,000
11 June	3,000,000	300,000
9 July	8,000,000	80,000,000
23 July	5,000,000	---
17 September	110,000	230,000,000
15 October	2,800,000	1,100,000
12 November	33,000,000,000	790,000,000
17 December	33,000,000	2,200,000

Table 12

Counts of total coliforms by MPN technique at Malvarrosa

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
23 January	7,000	4,900
19 February	23,000	23,000
27 March	7,000	8,000
2 May	7,000	8,000
28 May	>230,000	230,000
27 June	280	50
16 July	8,000	5,000
10 September	110,000	79,000
24 September	4,900	7,900
23 October	7,900	13,000
19 November	7,900	3,100
10 December	33,000	13,000

Table 13

Counts of total coliforms by MPN technique at Saler sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
23 January	35,000	220
19 February	4,900	7,900
27 March	30,000	13,000
2 May	3,000	900
28 May	13,000	11,000
27 June	240	50
16 July	500	350
10 September	13,000	4,900
24 September	920	94
23 October	33,000	26
19 November	79,000	540
10 December	33,000	540

Table 14

Counts of total coliforms by MPN technique at Perello

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
23 January	4,900	33
19 February	23,000	7,900
27 March	30,000	2,200
2 May	8,000	5,000
28 May	80,000	13,000
27 June	28,000	110
16 July	5,000,000	11,000
10 September	3,300,000	350,000
24 September	70,000	3,300
23 October	2,300	350
19 November	4,900	2,400
10 December	4,900	49

Table 15

Counts of total coliforms by MPN technique at Cullera sites

Sampling period (1990)	Counts of total coliforms MPN/100 ml	
	Site 1	Site 2
23 January	130,000	2
19 February	130,000	2,300
27 March	5,000	4
2 May	140,000	900
28 May	13,000	11
27 June	13,000	50
16 July	130,000	140
10 September	22,000	49
24 September	7,900	110
23 October	11,000	170
19 November	49,000	7
10 December	2,200	27

Table 16

Counts of Faecal coliforms by MPN technique and Membrane filtration resuscitation method at Puzol sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
15 January	34	79	100	600
5 February	790	920	800	770
5 March	3,000	1,600	5,000	2,000
17 April	4	4	18	20
14 May	5,000	220	4,000	670
11 June	50	30	300	310
9 July	900	500	600	220
23 July	130	50	200	120
17 September	540	130	700	500
15 October	350	110	700	300
12 November	540	49	130	120
17 December	3,300	2,300	3,300	3,100

Table 17

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Puig sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
15 January	28,000	17,000	18,000	22,000
5 February	13,000	3,300	24,000	6,000
5 March	3,000	3,000	3,000	3,000
17 April	280,000	28,000	300,000	30,000
14 May	500,000	30,000	500,000	20,000
11 June	170,000	110,000	290,000	130,000
9 July	280,000	3,000	140,000	10,000
23 July	5,000,000	22,000	11,000,000	30,000
17 September	460,000	130,000	540,000	120,000
15 October	22,000	13,000	51,000	16,000
12 November	490,000	79,000	240,000	20,000
17 December	33,000	1,100	50,000	2,000

Table 18

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Puebla de Farnals sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
15 January	70×10^4	46×10^2	148×10^4	30×10^2
5 February	49×10^4	13×10^2	28×10^4	40×10^2
5 March	35×10^4	30×10^2	70×10^4	50×10^2
17 April	70×10^3	13	14×10^4	30
14 May	80×10^4	50×10^2	54×10^4	33×10^2
11 June	28×10^5	13×10^3	34×10^5	33×10^3
9 July	14×10^7	90 x 10	24×10^7	20×10^2
23 July	23×10^7	22×10^3	26×10^7	42×10^3
17 September	35×10^7	22×10^4	10×10^7	20×10^5
15 October	35×10^5	79×10^2	10×10^6	16×10^3
12 November	18×10^9	17×10^4	10×10^9	86×10^4
17 December	33×10^6	17×10^3	48×10^6	16×10^3

Table 19

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Port Saplaya sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
15 January	94×10^4	33×10^2	20×10^5	125×10^2
5 February	13×10^4	94×10	50×10^3	90×10^2
5 March	50×10^3	30×10^3	80×10^3	46×10^3
17 April	11×10^4	90×10	20×10^4	11×10^2
14 May	23×10^4	22×10^2	55×10^4	50×10^2
11 June	13×10^5	13×10^4	22×10^5	19×10^4
9 July	17×10^5	13×10^6	30×10^5	30×10^6
23 July	23×10^5	----	26×10^5	---
17 September	11×10^3	28×10^6	20×10^3	---
15 October	14×10^5	79×10^4	26×10^5	21×10^5
12 November	49×10^6	33×10^6	20×10^6	40×10^6
17 December	70×10^4	35×10^4	10×10^5	40×10^4

Table 20

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Malvarrosa sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
23 January	1,100	700	1,300	440
19 February	7,900	7,900	19,000	18,000
27 March	1,600	2,300	2,500	2,000
2 May	2,300	2,200	3,300	3,500
28 May	>230,000	80,000	>100,000	100,000
27 June	170	9	435	60
16 July	2,300	900	5,000	1,500
10 September	49,000	23,000	40,000	29,000
24 September	2,300	3,300	3,000	4,000
23 October	3,300	4,900	4,000	5,000
19 November	4,900	2,300	10,000	4,000
10 December	7,900	3,300	16,000	3,000

Table 21

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Saler sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
23 January	1,700	17	4,000	80
19 February	1,700	350	3,500	1,300
27 March	1,600	1,600	6,000	2,000
2 May	900	300	1,900	660
28 May	3,000	3,000	4,200	4,600
27 June	50	4	100	10
16 July	80	110	280	220
10 September	920	540	3,000	2,300
24 September	130	5	500	60
23 October	13,000	2	24,000	20
19 November	17,000	170	39,000	460
10 December	7,900	70	12,000	300

Table 22

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Perello sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
23 January	1,100	8	2,000	40
19 February	3,300	2,300	8,000	5,600
27 March	3,000	110	3,800	380
2 May	3,000	1,100	2,000	2,600
28 May	11,000	1,700	25,000	4,400
27 June	8,000	50	16,000	90
16 July	1,100,000	2,300	1,500,000	3,000
10 September	460,000	110,000	2,100,000	220,000
24 September	17,000	1,600	40,000	4,000
23 October	540	49	3,000	70
19 November	1,700	920	18,000	200
10 December	1,100	5	8,000	16

Table 23

Counts of Faecal coliforms by MPN technique and membrane filtration resuscitation method at Cullera sites

Sampling period (1990)	Counts of Faecal coliforms MPN/100 ml			
	MPN technique		Resuscitation method	
	Site 1	Site 2	Site 1	Site 2
23 January	7,900	0	22,000	10
19 February	3,300	26	2,000	40
27 March	500	0	600	4
2 May	30,000	300	22,000	500
28 May	2,300	0	7,000	7
27 June	2,300	23	9,000	31
16 July	50,000	7	90,000	40
10 September	4,900	22	14,000	30
24 September	1,100	22	5,000	20
23 October	1,400	22	8,000	140
19 November	11,000	0	38,000	5
10 December	700	8	1,000	14

3.3 Faecal coliforms, MF resuscitation technique

The results of faecal coliforms counts using membrane filter resuscitation technique are shown in: table 16 (Puzol), table 17 (Puig), table 18 (Puebla de Farnals), table 19 (Port Saplaya), table 20 (Malvarrosa), table 21 (Saler), table 22 (Perello) and table 23 (Cullera). In general, counts obtained with MF technique were higher than MPN ones (table 26).

However the analysis of variance revealed no significant differences between the two procedures (table 27). Interestingly, at Cullera site 2 MF resuscitation technique was more sensitive than MPN method when levels of faecal pollution were low.

Verification of typical yellow colonies from the m-T7 agar is shown in table 28. The specificity with this medium for faecal coliforms was 80.3%.

3.4 Motile Aeromonads

The levels of motile aeromonads at each sampling area were:

Puzol: At sites 1 and 2 (table 29) aeromonads exhibited generally similar cell densities and similar fluctuations throughout the year. The highest aeromonads level at site 1 was $20x10^3$ cfu/100 ml and the lowest 25 cfu/100 ml. At site 2 the greatest count of aeromonads was $88x10^2$ cfu/100 ml and the lowest 15 cfu/100 ml.

Table 24

Correlation coefficients of motile aeromonads with faecal indicators at different sites (using log transformed data from 1990)

Sites	TC[a] - FC[b]		TC - MA[c]		FC - MA	
	r	P<	r	P<	r	P<
Puzol 1[g]	0.95	0.01[d]	0.57	n.s[f]	0.61	n.s
Puzol 2[h]	0.95	0.01	0.73	0.05[e]	0.73	0.05
Puig 1	0.92	0.01	0.31	n.s	0.43	n.s
Puig 2	0.91	0.01	-	n.s	0.19	n.s
Puebla de Farnals 1	0.99	0.01	0.05	0.01	0.74	0.05
Puebla de Farnals 2	0.95	0.01	0.76	0.05	0.79	0.01
Port Saplaya 1	0.90	0.01	0.69	0.05	0.74	0.05
Port Saplaya 2	0.97	0.01	0.71	0.05	0.85	0.01
Malvarrosa 1	0.96	0.01	0.76	n.s	0.51	n.s
Malvarrosa 2	0.98	0.01	0.58	0.01	0.74	0.05
Saler 1	0.93	0.01	0.79	0.01	0.81	0.01
Saler 2	0.95	0.01	0.86	0.01	0.79	0.01
Perello 1	0.99	0.01	0.88	0.05	0.73	0.05
Perello 2	0.97	0.01	0.74	0.01	0.84	0.01
Cullera 1	0.87	0.01	0.85	0.01	0.87	0.01
Cullera 2	0.85	0.01	0.86	n.s	0.51	n.s
			0.61			

a: Total coliforms;
b: Faecal coliforms;
c: Motile aeromonads;
d: significant values at 0.99 level;
e: significant values at 0.95 level;
f: not significant
g: site 1 is near from the sewage discharge area
h: site 2 is separated 200 meters from the first point

Table 25

Bacterial water quality at different sites using E.E.C. and Spanish guidelines for bathing waters[a]

Sites	Total coliforms		Faecal coliforms		Classi-fication[d]
	no of samples[b]	%	no of samples[c]	%	
Puzol 1	11	92	9	75	U
Puzol 2	12	100	11	92	G
Puig 1	1	8	0	-	U
Puig 2	2	17	1	8	U
Puebla de Farnals 1	0	-	0	-	U
Puebla de Farnals 2	4	33	4	33	U
Port Saplaya 1	0	-	0	-	U
Port Saplaya 2	2	17	2	17	U
Malvarrosa 1	8	67	3	25	U
Malvarrosa 2	7	58	3	25	U
Saler 1	5	25	8	67	U
Saler 2	9	75	11	92	U
Perello 1	5	25	4	33	U
Perello 2	9	75	9	75	U
Cullera 1	3	25	4	33	U
Cullera 2	12	100	12	100	E

a: The E.C.C. Directive for bathing waters specifies two levels of compliance for bacterial indicators:

- Total coliforms: I, "mandatory", when a count of 10,000 per 100 ml must not be exceeded for 95% of samples
  G, "guide", when a count of 500 per 100 ml must not be exceeded for 80% of samples
- Faecal coliforms: I, "mandatory:, when a count of 2,000 per 100 ml must not be exceeded for 95% of samples
  G, "guide", when a count of 100 per 100 ml must not be exceeded for 80% of samples;

b: no of samples with counts of total coliforms lower than 10,000/100 ml;
c: no of samples with counts of faecal coliforms lower than 2,000/100 ml;
d: Classification;
U: unfit;
G: good;
E: excellent.

Table 26

Comparison of faecal coliforms counts obtained by MF resuscitation method and MPN technique at all sampling sites

Sampling site	No of samplings[a]	
	Site 1	Site 2
Puzol	8	10
Puig	7	9
Puebla de Farnals	8	9
Port Saplaya	10	10
Malvarrosa	10	9
Saler	12	12
Perello	11	12
Cullera	10	12

a: Number of samplings with MF resuscitation faecal coliforms counts higher than MPN method (from a maximum of 12 samplings)

Table 27

Analysis of variance of the numbers of faecal coliforms at different sites showing the effect of MPN method and MF resuscitation technique

Sampling area	Site 1*			Site 2**		
	D.F.[a]	M.S.[b]	F-Snedecor[c]	D.F.	M.S.	F-Snedecor
Puzol	1	164	0.212	1	134	1.882
Puig	1	622	0.025	1	425	0.049
Puebla de Farnals	1	1.175	0.008	1	369	0.473
Port Saplaya	1	802	0.123	1	501	0.205
Malvarrosa	1	331	0.289	1	288	0.176
Saler	1	256	1.329	1	117	1.867
Perello	1	398	1.012	1	181	0.356
Cullera	1	334	1.717	1	31	2.661

*: site 1 is near from the sewage discharge area
**: site 2 is separated 200 meters from the first point
a: Degrees of freedom
b: M.S. : Mean square
c: F-Snedecor: Tabular F 0.05 and F 0.01 for 1 and 22 degrees of freedom are 4.30 and 7.95 respectively

Table 28

Verification of colonies observed on m-T7 agar

Culture medium	No. of colonies observed[a]	Typical colonies		Typical colonies	
		verified (TC)[b]	%	verified (FC)[c]	%
m-T7 agar	366	340	92.9	294	80.3

a: yellow colonies were counted as faecal coliforms;
b: verified as total coliforms;
c: verified as faecal coliforms.

Puig: the highest numbers of motile aeromonads were found at site 1 (table 30), with a maximum count of $83x10^5$ cfu/100 ml, while the counts at site 2 were lower, with a maximum count of $19x10^4$ cfu/100 ml.

Puebla de Farnals: at site 1 (table 31) aeromonads counts were greatest during summer and autumn with a peak in winter, the levels of aeromonads at this point were up to 10^5 cfu/100 ml. At site 2 aeromonad counts decreased 1,000 to 10,000 fold.

Port Saplaya: aeromonads values at site 1 (table 32) remained high all the year, the counts were up to 10^5 cfu/100 ml except the month of May. Site 2 showed low levels of motile aeromonads from January to May, except the month of March, while the counts increased from June to December due to the presence of a new sewage discharge point influencing this site.

Malvarrosa: at this sampling area aeromonad populations did not exhibit seasonal fluctuations (table 33). The levels of motile aeromonads were similar throughout the sampling period at sites 1 and 2. The highest aeromonads count at site 1 was $68x10^3$ cfu/100 ml and at site 2 $48x10^3$ cfu/100 ml.

Saler: at site 1 (table 34), aeromonad levels were found in the range of $97x10^3$ cfu/100 ml to 260 cfu/100 ml. At site 2, the aeromonad counts were similar, ranging from $26x10^3$ cfu/100 ml to 42 cfu/100 ml.

Perello: the highest recorded aeromonad counts for the year were at site 1 (table 35), approximately 10 to 100 times greater than site 2. At site 1, the results demonstrated a high variation in aeromonad levels ($20x10^2$ cfu/100 ml to $30x10^5$ cfu/100 ml). At site 2 the greatest aeromonad count was $26x10^4$ cfu/100 ml and the lowest 100 cfu/100 ml.

Cullera: at sites 1 and 2 (table 36) concentration of aeromonads was quite constant all the year around. At site 1 aeromonads were up to 10^3 cfu/100 ml with a maximum count of $20x10^4$ cfu/100 ml. At site 2 the levels of aeromonads decreased 10 to 1,000 fold.

Motile aeromonads were generally less abundant than total coliforms at Puzol, Puig, Puebla de Farnals, Port Saplaya, Malvarrosa and Cullera (site 2) sampling areas (table 37). However aeromonads were more abundant than faecal coliforms in all sampling areas (table 37).

The relationships between motile aeromonads and indicator organisms (total coliforms and faecal coliforms) were investigated at 8 sampling areas. Table 24 summarizes the values of correlation coefficients (r) and the confidence level (P) obtained between the concentrations of aeromonads and indicator organisms. Significant correlations were obtained between aeromonads and motile aeromonads in Puzol (site 2), Puebla de Farnals, Port Saplaya, Malvarrosa (site 2), Saler, Perello and Cullera (site 1) sampling areas.

The specificity of the mADA/0129 agar was determined by examination of 794 aeromonads typical colonies (table 38), the overall confirmation rate was 96.5% whereas 5.5% of the presumptively negative colonies were, in fact, motile aeromonads.

Table 29

Counts of motile Aeromonads by membrane filtration technique at Puzol sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
15 January	5,300	5,600
5 February	2,600	1,500
5 March	20,000	4,000
17 April	25	30
14 May	11,500	770
11 June	168	15
9 July	100	100
23 July	200	130
17 September	8,800	4,000
15 October	530	330
12 November	40	48
17 December	7,900	8,800

Table 30

Counts of motile Aeromonads by membrane filtration technique
at Puig sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
15 January	1,700	7,300
5 February	36,000	6,800
5 March	400,000	4,300
17 April	200,000	30,000
14 May	170,000	1,000
11 June	170,000	190,000
9 July	730,000	9,000
23 July	8,300,000	29,000
17 September	670,000	9,500
15 October	22,000	14,000
12 November	3,500	2,000
17 December	106,000	11,000

Table 31

Counts of motile Aeromonads by membrane filtration technique
at Puebla de Farnals sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
15 January	120,000	1,000
5 February	400,000	7,300
5 March	9,000,000	10,000
17 April	420,000	100
14 May	270,000	12,000
11 June	11,000,000	33,000
9 July	850,000,000	100
23 July	>1,000,000	23,000
17 September	136,000,000	2,240,000
15 October	1,200,000,000	20,000
12 November	2,300,000,000	10,400
17 December	970,000,000	9,100

Table 32

Counts of motile Aeromonads by membrane filtration technique
at Port Saplaya sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
15 January	3,190,000	5,700
5 February	1,060,000	6,000
5 March	140,000	130,000
17 April	970,000	10,000
14 May	20,000	1,000
11 June	2,400,000	550,000
9 July	270,000	960,000
23 July	6,200,000	---
17 September	100,000	---
15 October	22,200,000	2,300,000
12 November	100,000,000	470,000
17 December	16,000,000	630,000

Table 33

Counts of motile Aeromonads by membrane filtration technique
at Malvarrosa sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
23 January	3,000	3,400
19 February	68,000	33,000
27 March	11,000	220
2 May	2,200	3,100
28 May	>10,000	>10,000
27 June	1,200	30
16 July	1,000	1,000
10 September	32,000	48,000
24 September	4,000	5,000
23 October	400	600
19 November	1,000	200
10 December	9,000	1,000

Table 34

Counts of motile Aeromonads by membrane filtration technique at Salar sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
23 January	59,000	5,000
19 February	97,000	26,000
27 March	72,000	19,000
2 May	490	440
28 May	36,000	21,800
27 June	480	160
16 July	260	200
10 September	33,000	3,000
24 September	4,500	100
23 October	78,000	42
19 November	70,000	1,600
10 December	78,000	2,800

Table 35

Counts of motile Aeromonads by membrane filtration technique at Perello sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
23 January	7,300	1,300
19 February	40,000	4,000
27 March	110,000	7,200
2 May	22,000	8,800
28 May	17,000	8,000
27 June	92,000	500
16 July	410,000	5,500
10 September	3,000,000	260,000
24 September	140,000	14,000
23 October	2,000	100
19 November	56,000	1,100
10 December	260,000	130

Table 36

Counts of motile Aeromonads by membrane filtration technique at Cullera sites

Sampling period (1990)	motile aeromonads cfu/100 ml	
	Site 1	Site 2
23 January	180,000	0
19 February	20,000	290
27 March	9,800	200
2 May	200,000	400
28 May	15,500	22
27 June	45,000	23
16 July	130,000	10
10 September	32,000	14
24 September	17,000	41
23 October	12,000	104
19 November	83,000	9
10 December	2,000	2

Table 37

Comparison of motile *Aeromonas* levels and bacterial indicators densities (MPN method) at different sampling sites[1]

Sampling area	MA[a]/TC[b]	MA/FC[c]
Puzol site 1	5/12	10/12
Puzol site 2	5/12	10/12
Puig site 1	0/12	6/12
Puig site 2	0/12	8/12
Puebla de Farnals (site 1)	5/12	6/12
Puebla de Farnals (site 2)	4/12	8/12
Port Saplaya (site 1)	4/12	10/12
Port Saplaya (site 2)	4/10	7/10
Malvarrosa (site 1)	3/12	6/12
Malvarrosa (site 2)	1/12	7/12
Saler (site 1)	8/12	11/12
Saler (site 2)	9/12	12/12
Perello (site 1)	8/12	11/12
Perello (site 2)	6/12	12/12
Cullera (site 1)	9/12	12/12
Cullera (site 2)	3/12	9/12

1: Number of samples with motile aeromonad counts higher than bacterial indicators densities

a: Motile aeromonads;
b: Total coliforms;
c: Faecal coliforms

Table 38

Verification of colonies observed on mADA/0129 agar

No. of colonies tested	Typical colonies		Non typical colonies			
	Verified as MA[a](%)	Non MA(%)	MA(%)	CF[b](%)	PS[c](%)	Ox-[d](%)
794	767(96.5)	27(3.5)				
271			15(5.5)	41(15.5)	154(56)	51(19)

a: motile aeromonads;
b: coliforms;
c: *Pseudomonas* spp.;
d: oxidase negative non identified.

Biochemical reactions were recorded for 759 motile aeromonad strains (table 39). Biotyping distinguished the species *A. caviae, A, hydrophila* and *A. sobria*. The most frequent species identified were *A. caviae* (79.8%) followed by *A. hydrophila* and *A. sobria* (5.3%).

3.5 *Campylobacter*

Sample sizes filtered to recover campylobacters ranged from 100 ml (sites 1) to 1,000 ml (sites 2).

A total of 192 samples was tested. The isolation frequency of campylobacters was higher from the polluted areas than from the unpolluted areas. Thirteen per cent (25/192) of samples yielded campylobacters (table 40). Of the 16 sites examined, campylobacters were isolated from 8 sites. The frequency of isolation of campylobacters from each positive site varied from 8% (1/12) to 58% (7/12).

Campylobacters were more frequently isolated from water samples taken at Saler (site 1) and Port Saplaya (site 1) in winter and spring than in corresponding samples taken in summer or autumn. At Puebla de Farnals and Perello sites *Campylobacter* presence not exhibited seasonal fluctuation, and was independent of environmental temperatures.

At Perello (site 1) campylobacters were detected throughout the whole of the sampling period although not equally. At this site campylobacters were isolated when faecal coliforms exceeded 1,000/100 ml. *Campylobacter* presence was detected at sites 2 of Perello and Puebla de Farnals and this fact is remarkable because these sites are separated 200 meters from sewage discharge area. The data obtained indicate that there is little relationship between *Campylobacter* recovery and faecal coliform densities.

A total of 21 *Campylobacter* strains was subtyped. The results are summarized in table 41.

Table 39

Motile aeromonads species isolated on mADA/0129 agar
at different sites

Sampling site	No. of strains identified (759)			
	A. caviae	*A. hydrophila*	*A. sobria*	*Aeromonas* sp
Puzol 1	42	8	1	2
Puzol 2	31	7	1	0
Puig 1	36	9	7	2
Puig 2	45	5	4	2
Puebla de Farnals 1	31	6	2	2
Puebla de Farnals 2	21	7	1	1
Port Saplaya 1	39	4	5	4
Port Saplaya 2	32	3	1	2
Malvarrosa 1	52	3	0	2
Malvarrosa 2	31	2	1	2
Saler 1	51	6	5	1
Saler 2	40	4	6	4
Perello 1	46	5	2	3
Perello 2	44	1	1	1
Cullera 1	33	12	3	1
Cullera 2	32	2	0	1
Total (%)	606(79.8)	83(10.9)	40(5.3)	30(3.9)

1: Sites located near sewage discharge area;
2: Sites separated 200 meters from sewage discharge area

Table 40

Campylobacter occurrence at different sites (sampling year 1990)

Sampling area	Date												
	23/01	19/02	27/03	2/05	28/05	27/06	16/07	10/09	24/09	23/10	19/11	10/12	Total[a]
Malvarrosa 1	-[b]	-	-	-	-	-	-	-	-	-	-	-	0
Malvarrosa 2	-	-	-	-	-	-	-	-	-	-	-	-	0
Saler 1	-	-	+[c]	-	+	-	-	-	-	-	-	-	0
Saler 2	-	-	-	-	-	-	-	-	-	-	-	-	0
Perello 1	-	+	-	+	+	-	-	+	+	-	+	+	7
Perello 2	-	-	-	-	-	-	-	+	+	-	-	-	2
Cullera 1	-	-	-	+	-	-	-	-	-	+	-	-	2
Cullera 2	-	-	-	-	-	-	-	-	-	-	-	-	0
	15/01	5/02	5/03	17/04	14/05	11/06	9/07	23/07	17/09	15/10	12/11	17/12	
Puzol 1	-	-	-	-	-	-	-	-	-	-	-	-	0
Puzol 2	-	-	-	-	-	-	-	-	-	-	-	-	0
Puig 1	-	-	-	-	-	-	-	-	-	-	-	+	1
Puig 2	-	-	-	-	-	-	-	-	-	-	-	-	0
Puebla de Farnals 1	-	+	+	-	+	+	+	-	-	-	-	-	5
Puebla de Farnals 2	-	+	-	-	-	-	-	-	-	-	-	-	1
Port Saplaya 1	+	+	+	+	-	-	-	-	-	-	-	+	5
Port Saplaya 2	-	-	-	-	-	-	-	-	-	-		-	0

a: No. of samples positives; b: Occurrence negative; c: Occurrence positive

Table 41

Campylobacter species identified at different sites

Sampling area	Number of isolates	*C. jejuni*	*C. coli*	*Campylobacter* sp
Puig (site 1)	1	0	0	1
Puebla de Farnals (site 1)	5	2	3	0
Puebla de Farnals (site 2)	1	0	0	1
Port Saplaya (site 1)	5	2	2	1
Saler (site 1)	2	0	2	0
Perello (site 1)	7	0	5	2
Perello (site 2)	2	0	1	1
Cullera (site 1)	2	0	2	0
Total (%)	25	4(16%)	15(60%)	6(24%)

6 *Campylobacter* strains has been not identified due to interference during the culture procedure. *Campylobacter coli* strains were more frequently isolated than *Campylobacter jejuni* strains from all water samples. *Campylobacter jejuni* strains were isolated from water samples taken in winter and spring.

3.6 Temperature

The water temperatures measured throughout the year are indicated in Table 42. The highest temperature was 28E C and the lowest 9E C.

Table 42

Water temperature through the year 1990 (E C)

Winter			Spring			Summer			Autumn		
Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
9	16	12.4	12	24	20.4	26	28	26.5	12	25	18.7

4. DISCUSSION

4.1 Injured faecal coliforms

Since the 13th edition of Standard Methods (APHA 1971), the membrane filter (MF) has been accepted as an alternative method for the detection and enumeration of faecal organisms in water. Nevertheless the phenomenon of MF techniques producing lower counts than the MPN method in the bacteriological examination of certain aqueous environments has been recognized and is well documented (Braswell and Hoadley 1974, Maxey 1970). It has

been demonstrated that membrane filter media commonly used for water analysis, containing aniline blue or more than 0.05% bile salts or deoxycholate were highly toxic to injured coliforms (LeChevallier *et al.* 1983). The majority of selective media used to isolated gram negative bacteria recovered 30% or less of the injured coliforms (LeChevallier *et al.* 1984). Increasing attention is being given to the recovery of injured indicator bacteria (Lin 1976, McFeters *et al.* 1982, LeChevallier *et al.* 1984, Camper and McFeters 1979).

Faecal coliforms recovery comparisons from the MPN and m-T7 MF procedures were conducted at sixteen sites. The results obtained in this study showed that m-T7 MF technique was superior to MPN procedure, although no differences at the 95% confidence level were found at any of the sixteen sites examined; therefore, both methods apparently measure the same population. It is appropriate to mention that the MPN estimation overestimate the true bacterial density from a given test sample (El-Shaarawi and Pipes 1982).

Interestingly, the results reported at Cullera site 2 indicate that the m-T7 method was more sensitive than the MPN method in seawater samples with low level of faecal pollution. The lower results of MPN procedure were probably due to the inhibitory action of sodium lauryl sulfate (Hill 1967), and the absence of gas production in the presumptive portion of the MPN test because the enzyme formic hydrogen lyase (which produces gas from formic acid) may be impaired or not induced in environmental stresses cells (Olson 1978). Although the MPN procedure provides an environment in which injured cells may repair themselves (24 to 40h of incubation at a lower temperature, 35E C, in a nonselective lactose broth medium) before being subjected to a higher temperature (44.5E C) and a more selective medium (EC broth) (Stuart *et al.* 1977). The physiological consequences of injured cells are leakage of intracellular material, including macromolecules, amino acids and specific ions; increased lipid synthesis; altered nutrient transport; increased sensitivity to NaCl; and uncoupling of oxidative phosphorylation (Beauchat 1978).

The specificity of the m-T7 agar obtained in this study for marine waters was 80.3%. LeChevallier *et al.* (1984) found that the presence of faecal coliforms on m-T7 agar was confirmed in 89% of presumptive colonies isolated from chlorinated and no chlorinated waters.

The m-T7 MF technique has several distinct advantages. The enumeration of faecal coliforms is completed within 24 hours, whereas the MPN procedure requires 3 days to obtain results for faecal coliforms. There are no overlay procedures, nor does the filter need to be transferred. It is more precise than the MPN method. One disadvantage is that an incubator is required capable of adjusting temperatures after an 8-hour interval. However this problem can be avoided by using a temperature-programmed incubator that makes the change from 37E to 44.5E C (LeChevallier *et al.* 1984).

The results reported here indicate that m-T7 method produces data comparable to those of the MPN procedure when used to enumerate faecal coliforms in marine recreational waters.

4.2 Motile *Aeromonas*

Motile aeromonads are widely distributed in aquatic environments (Joseph *et al.* 1988). Although many studies on the recovery of clinical *Aeromonas* spp. have been reported to date, environmental approaches are scarce.

In this study aeromonads were isolated in the sixteen sites analyzed but at different densities. The highest levels of aeromonads were detected at sites 1 of Puebla de Farnals and Port Saplaya, with densities up to 10^5/100 ml. These zones showed also high numbers of faecal indicators. At sites 2 of Puig, Puebla de Farnals, Port Saplaya, Perello and Saler aeromonads densities decreased. The numbers of aeromonads detected at sites 1 and 2 of Malvarrosa were similar. The aeromonads level was low at Puzol zone. These data indicated that densities of aeromonads in seawater depend on the degree and origin of sewage pollution. Rippey and Cabelli (1985) demonstrated that the rate of growth of aeromonads is increased by raising the level of nutrients, particularly phosphates, in water. Aeromonads are not fastidious in their growth requirements and simple organic compounds can serve as growth substrates (Van der Kooij *et al.* 1980).

The concentration of aeromonads exceeded those of faecal coliforms in all sampling areas monitored. Similar results were obtained by other investigators in surface waters and sewage (Kaper *et al.* 1981, Rippey and Cabelli 1980, Seidler *et al.* 1980, Araujo *et al.* 1990, Larsen and Willeberg 1984). The use of the linear correlation coefficient is helpful in establishing the relationship between aeromonads and indicator microorganisms. Positive correlations were found between aeromonads densities and bacterial indicators at sites 1 and 2 of Puebla de Farnals, Port Saplaya, Saler and Perello. At sites 1 of Puzol and Malvarrosa there was no correlation between aeromonads levels and bacterial indicators but at sites 2 of these zones the correlation was positive. At site 1 of Cullera there was a significant correlation between the numbers of aeromonads and bacterial indicators however at site 2, with low faecal pollution, there was no correlation. At sites 1 and 2 of Puig there was no relationship between the densities of total and faecal coliforms and aeromonads. This can be due to the fact that this area is influenced by sewage discharges of a brewery and it has been observed that waters receiving carbohydrate rich industrial effluents *Klebsiella* spp. is found in very high densities (Dufour and Cabelli 1976), consequently the faecal coliform population at Puzol sites could be non exclusively from faecal origin. *Aeromonas* spp. have a close relationship with indicator organisms of faecal pollution in habitats where the organic matter is of faecal origin but not in those habitats where organic matter is of a different origin (Araujo *et al.* 1989b, 1990). The values of correlation coefficient between aeromonads and bacterial indicators must be interpreted in relation to the source and inactivation kinetics and not in terms of a functional dependence. We agree other authors (Araujo *et al.* 1990) that the health risk from aeromonads in bathing waters cannot always be estimated by reference to faecal indicators and therefore aeromonads need to be monitored as well.

Aeromonads population not exhibited seasonal fluctuations in the majority of sites analyzed and was independent of environmental temperatures. Nevertheless aeromonads levels increased at Puebla de Farnals for samples collected in summer and autumn when the greatest isolation of faecal coliform occurred. Other investigators (Araujo *et al.* 1988, Hazen *et al.* 1978) found that the isolation of aeromonads was not significantly related to the environmental temperatures. Although Kaper *et al.* (1981) found a significant effect of temperature.

Preliminary speciation based on a limited number of biochemical tests (acid and gas production from glucose and esculine hydrolysis was performed). *Aeromonas caviae* seems to be higher in the waters studied by us than *A. hydrophila* and *A. sobria*. Arribas *et al.* (1987) detected *A. caviae* as predominant in polluted marine waters. However other authors (Seidler *et al.* 1980) found *A. hydrophila* to be majority.

The AH medium seems to be useful for a presumptive and fast identification of motile *Aeromonas*. These results are consistent with those reported by Toranzo *et al.* (1986).

The specificity achieved by the use of mADA/0129 agar was excellent, out of 794 presumptively positive colonies biochemically identified, 3.5% were false positive. The rate of false negative colonies was 5.5%. A typical colonies were predominantly identified as coliforms and *Pseudomonas*. The specificity criterion (Levin and Cabelli 1972) (that no more than 10% false positives and 10% false negatives will be obtained) was satisfied. Therefore, it may be assumed that verification of typical colonies will not be required routinely.

The mADA/0129 MF method allows a rapid and reliable enumeration of *Aeromonas* spp. from marine recreational waters.

The densities of aeromonads found in this study may have implications in the significance of gastrointestinal disease associated with this bacteria. Nevertheless the specific public health significance of these findings is unknown, since the minimum infection dose for aeromonads has not been determined. Several reports have demonstrated wound infections caused by *Aeromonas* and such infections have been linked circumstantially with recreational and other activities known to contain *Aeromonas* (Fulghum *et al.* 1978, Hanson *et al.* 1977, Joseph *et al.* 1979). The potential public health significance of large numbers of aeromonads in aquatic environments cannot be ignored.

4.3 *Campylobacter*

Campylobacter enteritis in the last few years have been found to cause as much enteric disease in man as *Salmonella* and *Shigella* and thus has emerged as an important human intestinal pathogen (Smibert 1984). Water is potentially an important reservoir of the thermophilic campylobacters and is an established vehicle for the transmission of these organisms to man and domestic animals (Bolton *et al.* 1987). Notwithstanding there is few surveys of *Campylobacter* presence in seawater.

In the present study *Campylobacter* isolates were never isolated from seawater samples of 100 ml volume with levels of faecal coliforms less than 1,000/100 ml. Similar findings were found in river water samples by Stelzer *et al.* (1988). The higher isolation frequency of campylobacters at Perello (site 1) in comparison with Puebla de Farnals (site 1) and Port Saplaya (site 1) was not explained by higher faecal contamination in the former ones. It has been observed in freshwaters that there is no obvious relationship between the numbers of total and faecal coliforms and the occurrence of *Campylobacter* spp. (Carter *et al.* 1987, Taylor *et al.* 1983, Bolton *et al.* 1987, Martikainen *et al.* 1990). It seems that faecal bacterial indicators may not be good predictors for the presence of *Campylobacter* spp. in seawater.

In our study area the sites analyzed for *Campylobacter* presence were influenced by sewage discharges from different origin. Jones *et al.* (1990c) detected high numbers of campylobacters in seawater of Morecambe Bay (Lancaster) and they observed that *Campylobacter* presence was presumably due to the discharge of sewage into the sea. It has been found that sewage effluent is the main source of thermophilic campylobacters in surface waters (Bolton *et al.* 1987, Arimi *et al.* 1988, Holler 1988). Potential sources of campylobacters in the sewage system include human faeces, industrial waste, farm wastes and faeces from household pets (Fricker and Park 1989). The occurrence of campylobacters in surface waters also depend on the campylobacter carriage of animals such as seagulls (Fricker *et al.* 1983).

In the present study it has been observed a seasonal relationship between *Campylobacter* presence and temperature of seawater, although not all the sampling sites showed the same seasonality. At Saler (site 1) and Port Saplaya (site 1) campylobacters were isolated at lower temperatures. At Puebla de Farnals (site 1) and Perello (site 1) campylobacters were recovered throughout the year. Other workers (Bolton *et al.* 1987, Carter *et al.* 1987, Martikainen *et al.* 1990, Jones *et al.* 1990c) have also found a seasonal occurrence of *Campylobacter* spp. in surface waters. Rollins and Colwell suggested that campylobacters maintain their culturability in stream water for longer periods at low temperatures and that culturability declines as water temperatures increase. Nevertheless temperature may partly explain the occurrence of campylobacters in surface waters. The degree to which surface waters become contaminated with the bacteria could be another factor influencing seasonal variations noted in the occurrence of *Campylobacter* spp. in water (Carter *et al.* 1987). Bolton *et al.* (1987) indicated that low numbers of thermophilic campylobacters occur in river during the summer because of the biocidal effects of sunlight. Experiments on the effects of light showed that campylobacters in sewage effluent and seawater were eliminated within 60 and 30 min of daylight respectively but survived for 24h in darkness (Jones *et al.* 1990c). There is also evidence that *Campylobacter jejuni* is more sensitive to U.V. radiation than some other pathogenic bacteria (Butler *et al.* 1987). Carter *et al.* (1987) observed that the amount of rainfall and runoff could also be an important factor in seasonal variation occurrence of campylobacters. In seawater the occurrence of *Campylobacter* spp. may be influenced by any combination of the factors discussed above and by factors to be determined.

The dominant *Campylobacter* species isolated from sampling sites analyzed was *C. coli*. Several authors (Bolton *et al.* 1987, Stelzer *et al.* 1989, Rosef *et al.* 1987, Martikainen *et al.* 1990) isolated from freshwaters more frequently *C. jejuni* strains than *C. coli* strains. Although *C. coli* has been reported to be more prevalent in other surveys of natural waters (Pearson *et al.* 1985). Fricker and Park (1989) observed that certain strains of campylobacters will survive better in river water than other strains. It is possible that the potential differences in survival may be reflected in distribution of biotypes obtained in seawater. The ability to recover campylobacters is influenced by technique, the physiological state of the organisms, and the number of cells present. The antibiotic rifampicin was present in Preston enrichment and isolation media evaluated in the present study, thus perhaps exerting a detrimental effect on recovery of campylobacters from seawater.

Humphrey and Cruickshank (1985) indicated that for environmental samples where sublethally injured campylobacters may be present it would seem advisable to use medium

that does not contain rifampicin. However *Campylobacter* damaged cells are less sensitive to antibiotics and other selective pressures at 37E C (Humphrey 1986). In our study we have followed the recommendations of Humphrey (1989) to maximize the recovery of sublethally injured campylobacters and we have included a quenching oxygen agent in the media and preincubated Preston enrichment medium at 37E C for up to 4h.

5. CONCLUSIONS

Injured faecal coliforms

In the marine recreational waters analyzed by us, it has been demonstrated that m-T7 MF method can be used for the recovery of injured faecal coliforms. This technique has been shown to be as efficient as MPN method.

Motile aeromonads

The mADA/0129 procedure has satisfied the criteria for a primary, selective and differential method for the enumeration of motile aeromonads from polluted seawaters. We agree other authors (Araujo *et al.* 1989b) that the exclusive use of the faecal bacterial indicators underestimate the risk of infection posed by opportunistic pathogens like *Aeromonas* spp. and we consider it advisable to monitor these bacteria in recreational waters where the risk of direct infection to humans is high.

Epidemiological studies are needed to determine the relationship between cases of *Aeromonas*-associated diarrhoea and the densities of these organisms in marine recreational waters.

In the absence of more definite proof of the public health significance (or insignificance), it is advisable to prevent exposure of the bathers to high densities of aeromonads by limiting the numbers and the regrowth possibilities of these organisms in seawater.

Campylobacter

The present study has demonstrated that marine recreational waters contain a variety of campylobacters and when environmental contamination does occur, the effect of sewage effluent discharged into marine waters is important.

Knowledge of the survival characteristics of thermophilic campylobacters in seawater if of prime importance.

Further studies are needed to identify possible indicator organisms which could be used to predict the likely presence of *Campylobacter* spp. in sewage polluted seawater. Data from this investigation provide information of seasonal occurrence of thermophilic campylobacters in marine waters and identification of the periods of greatest health hazard. However epidemiological studies are needed to elucidate the public health significance of campylobacters in marine recreational waters.

We agree with other authors (Fricker 1987) that there is need for more research to develop sensitive and universally accepted enrichment procedures particularly for sample in

which sublethally damaged campylobacters are likely to be present. Also are necessary additional studies to evaluate a blood-free medium for laboratories not routinely using blood.

6. REFERENCES

General

APHA/AWWA/WPCF (1971) *Standard Methods for the Examination of water and Wastewater, 13th ed.* American Public Health Association, Washington.

APHA/AWWA/WPCF (1985) *Standard Methods for the Examination of Water and Wastewater, 16th ed.* American Public Health Association, Washington.

CHEESBROUGH, M. (1984) *Medical Laboratory Manual for Tropical Countries.* Tropical Health Technology, Butterworths, England.

DIRECTIVE C.E.E. (1976) - Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC). *Official Journal of the European Communities L31, 5 February 1976.*

REAL DECRETO 734/1988, de 1 de Julio, por el que se establecen normas de calidad de las aguas de baño. BOE No. 167 de 13 de Julio y BOE No. 169 de 15 de Julio.

WASHINGTON J.A. (1985) *Laboratory Procedures in Clinical Microbiology.* Springer-Verlag, New York.

Injured faecal coliforms

BEAUCHART, L.R. (1978) Injury and the repair of gram-negative bacteria with special consideration of the involvement of the cytoplasmic membrane. In: *Advances in Applied Microbiology, Vol 23,* D. Perlman (ed.). Academic Press, New York, pp. 219.

BISONNETTE, G.D., JEZESKI, J.J., McFETERS, G.A. and STUART, D.G. (1975) Evaluation of recovery methods to detect coliforms in water. *Appl. Microbiol.* **33:** 590-595.

BRASWELL, J.R. and HOADLEY, A.W. (1974) Recovery of *E. coli* from chlorinated secondary sewage. *Appl. Microbiol.* **28:** 328-329.

BRODSKY, M.H. and SCHIEMANN, D.A. (1975) Influence of coliform source on evaluation of membrane filters. *Appl. Microbiol.* **30:** 727-730.

CAMPER, A.K. and McFETERS, G.A. (1979) Chlorine injury and the enumeration of waterborne coliform bacteria. *Appl. Environ. Microbiol.* **37:** 633-641.

CARLUCCI, A.F., SCARPINO, A.V. and PRAMER, D. (1959) Factors affecting the survival of bacteria in seawater. *Appl. Microbiol.* **7:** 388-392.

CLARK, J.A. (1980) The influence of increasing numbers of non-indicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. *Can. J. Microbiol.* **26:** 827-832.

DUFOUR, A.P., STRICKLAND, E.R. and CABELLI, V.J. (1981) Membrane filter method for enumerating *Escherichia coli*. *Appl. Environ, Microbiol.* **41:** 1152-1158.

EL SHAARAWI, A.H. and PIPES, W.O. (1982) Enumeration and statistical inferences. In: *Bacterial Indicators of Pollution,* W.O. Pipes (ed.), pp. 44-66. CRC Press, Inc. Boca Raton, Florida.

FRYT, M.S. (1979) Effects of turbidity on microbiological analysis. In: *Proc. Of the AWWA Water Quality Tech. Conf.,* pp. 287-300. American Water Works Association, Philadelphia.

GAMESON, A.L.H. (1981) Bacterial pollution of bathing beaches. In: *Water Research Topics,* I.M.Ch. Lamont (ed.), vol.1, pp. 101-110. Ellis Horwood, Chichester.

GREEN, B.L., CLAUSEN, E.M. and LITSKY, W. (1977) Two temperature membrane filter method for enumerating faecal coliform bacteria from chlorinated effluents. *Appl. Environ. Microbiol.* **33:** 1259-1264.

HILL, M.N. (1967) Action of bile salts on bacterial cell walls. *Nature* **214:** 1152-1154.

LeCHEVALLIER, M.W., CAMERON, S.C. and McFETERS, G.A. (1983) New medium for improved recovery of coliform bacteria from drinking water. *Appl. Environ. Microbiol.* **45:** 484-492.

LeCHEVALLIER, M.W., JAKANOSKI, P.E., CAMPER, A.K. and McFETERS, G.A. (1984) Evaluation of m-T7 agar as a faecal coliform medium. *Appl. Environ. Microbiol.* **48:** 371-375.

LIN, S.D. (1976) Membrane filter method for recovery of faecal coliforms in chlorinated sewage effluents. *Appl. Environ. Microbiol.* **32:** 547-552.

LORENZ, R.C. and TUOVINEN, O.H. (1979) Inhibition by various membrane filters of *Escherichia coli* colony development. *Microb. Lett.* **12:** 23-30.

MAXEY, R.B. (1970) Non-lethal injury and limitations of recovery of coliform organisms on selective media. *J. Milk Food Technol.* **33:** 445-448.

McFETERS, G.A. and STUART, D.G. (1972) Survival of coliform bacteria in natural waters: field and laboratory studies with membrane filter chambers. *Appl. Microbiol.* **24:** 805-811.

McFETERS, G.A., CAMERON, S.C. and LeCHEVALLIER, M.W. (1982) Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. *Appl. Environ. Microbiol.* **43:** 97-103.

OLSON, B.H. (1978) Enhanced accuracy of coliform testing in seawater by a modification of the most probable number method. *Appl. Environ. Microbiol.* **36:** 438-444.

PIKE, E.B. and RIDGWAY, J.W. (1985) The microbiological implications in European Community Directive related to the water cycle. *J. Appl. Bacteriol. Symp.,* pp. 105-126.

REASONER, D.J., BLANNON, J.C. and GELDREICH, E.E. (1979) Rapid seven-hour faecal coliform test. *Appl. Environ. Microbiol.* **38:** 229-236.

STANFIELD, G., ROBINSON, J.A. and STANFIELD, J.P. (1977) *Investigations into membrane filtration procedure for determining the total coliform population of seawater samples.* Water Res. Tech. Report TR57 Medmenham Water Res. Ctr.

STUART, D.G., McFETERS, G.A. and SCHILLINGER, J.E. (1977) Membrane filter technique for the quantification of stressed faecal coliforms in the aquatic environment. *Appl. Environ. Microbiol.* **34:** 42-46.

TOBIN, R.S. and DUTKA, B.J. (1977) Comparison of the surface structure, metal binding and faecal coliform recoveries of nine membrane filters. *Appl. Environ. Microbiol.* **34:** 69-79.

ZASKE, S.K., DOCKINS, W.S. and McFETERS, G.A. (1980) Cell envelope damage in *Escherichia coli* caused by short term stress in water. *Appl. Environ. Microbiol.* **40:** 386-390.

Motile *aeromonas*

ALONSO, J.L. and GARAY, E. (1989) Two membrane filter media (mADA/0129 and mSA/0129 agars) for enumeration of motile *Aeromonas* in seawater. *Zbl. Bakt. Hyg., I. Abt. orig.* B **161:** 482-497.

ALONSO, J.L., AMOROS, I and BOTELLA, M.S. (1990) *Enumeration of motile Aeromonas in Valencia coastal membrane filters.* IAWPRC Health-related Water Microbiology Symp., Tubingen.

ALTWEGG, M., STEIGERWALT, A.G., ALTWEGG-BISSIG, R., LÜTHY-HOTTENSTEIN, J., and BRENNER, D.J. (1990) Biochemical identification of *Aeromonas* genospecies isolated from humans. *J. Clin. Microbiol.* **28:** 258-264.

AMOROS, I., ALONSO, J.L. y BOTELLA, M.S. (1989) Presencia de *Aeromonas* moviles resistentes a antibioticos en aguas costeras de Puebla de Farnals (Valencia). *XII Cong. Nac. Microbiol. Resumenes Comunic.* Vol.II. Pamplona.

ARAUJO, R.M., ARRIBAS, R.M., LUCENA, F. and PARES, R. (1988) Distribution of mesophilic aeromonads in temperate aquatic habitats: relationship with faecal indicator parameters. *IAWPRC Newsletter* **13:** 37.

ARAUJO, R., FRIAS, J., RIBAS, F.R., HUGHET, J.M., RIBAS, F. y LUCENA, F. (1989a) Comparacion de tres medios de cultivo selectivos para el recuento de *Aeromonas* moviles en aguas de diverso tipo. *XII Cong. Nac. Microbiol. Pamplona. Resumenes Comunic.* Vol.II.

ARAUJO, R.M., ARRIBAS, R.M., LUCENA, F. and PARES, R. (1989b) Relation between *Aeromonas* and faecal coliforms in fresh waters. *J. Appl. Bacteriol.* **67:** 213-217.

ARAUJO, R.M., PARES, R. and LUCENA, F. (1990) The effect of terrestrial effluents on the incidence of *Aeromonas* spp. in coastal waters. *J. Appl. Bacteriol.* **69:** 439-444.

ARRIBAS, R.M., ARAUJO, R.M. and PARES, R. (1987) Diferencias entre las poblaciones de *Aeromonas* presentes en distintos tipos de aguas contaminadas. *Proc. XI Nac. Cong. Microbiol.* Vol.II, pp. 764-765. Ed. Soc. Española de Microbiologia.

BARER, M.R., MILLERSHIP, S.E. and TABAQCHLI, S. (1986) Relationship of toxin production to species in the genus *Aeromonas*. *J. Med. Microbiol.* **22:** 303-309.

BUCHANAN, R.L. and PALUMBO, S.A. (1985) *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: a review. *J. Food Saf.* **7:** 15-29.

BURKE, V., ROBINSON, J., BEARNAN, J. GRACEY, M., LESMANA, M., ROCKHILL, R., ECHEVARRIA, P. and JANDA, M. (1983) Correlation of enterotoxicity with biotype in *Aeromonas* spp. *J. Clin. Microbiol.* **18:** 1196-1200.

BURKE, V., ROBINSON, J., GRACEY, M., PETERSON, D. and PARTRIDGE, K. (1984) Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. *Appl. Environ. Microbiol.* **48:** 361-366.

BURKE, V., COOPER, M. and ROBINSON, J. (1986) Haemagglutination patterns of *Aeromonas* spp. related to species and source of strains. *Aut. J. Experiment. Biol. Med. Sci.* **64:** 563-570.

CALLISTER, S.M. and AGGER, W.A. (1987) Enumeration and characterization of *Aeromonas hydrophila* and *Aeromonas caviae* isolated from grocery store produce. *Appl. Environ. Microbiol.* **53:** 249-253.

CARNAHAN, A., HAMMONTREE, L., BOURGEOIS, L. and JOSEPH, S.W. (1990) Pyrazinamidase activity as a phenotypic marker for several *Aeromonas* spp. isolated from clinical specimens. *J. Clin. Microbiol.* **28:** 391-392.

CUNLIFFE, D.A. and ADCOCK, P. (1989) Isolation of *Aeromonas* spp. from water by using anaerobic incubation. *Appl. Environ. Microbiol.* **55:** 2138-2140.

DAILY, O.P., JOSEPH, S.W., COOLBAUGHM, J.C., WALKER, R.I., MERREL, B.R., ROLLINS, D.M., SEIDLER, R.J., COLWELL, R.R. and LISSNER, C.R. (1981) Association of *Aeromonas sobria* with human infection. *J. Clin. Microbiol.* **13:** 769-777.

DIAZ, A. and VELASCO, A.C. (1987) Enteropatogenicidad de *Aeromonas* spp. *Rev. Esp. Microbiol. Clin.* **2:** 11-16.

DUFOUR, A.P. and CABELLI, V.J. (1976) Characteristics of *Klebsiella* from textile finishing plant effluents. *J.W.P.C.F.* **48:** 872-879.

FULGHUM, D.D., LINTON, W.J.Jr and TAPLIN, D. (1978) Fatal *Aeromonas hydrophila* infection due to *Aeromonas hydrophila*. *J. Am. Med. Assoc.* **238:** 1053-1054.

HANSON, P.G., STANDRIDGE, J., JARETT, F and MAKI, D.G. (1977) Freshwater wound infection due to *Aeromonas hydrophila*. *J. Am. Med. Assoc.* **238:** 1053-1054.

HAVELAAR, A.H., DURING, M. and VERSTEEGH, J.F.M. (1987) Ampicillin-dextrin agar medium for the enumeration of *Aeromonas* species in water by membrane filtration. *J. Appl. Bacteriol.* **62:** 279-287.

HAVELAAR, A.H. and VONK, M. (1988) The preparation of ampicillin dextrin agar for the enumeration of *Aeromonas* in water. *Lett. Appl. Microbiol.* **7:** 169-171.

HAZEN, T.C., FLIERMANS, C.B., HIRSH, R.F. and ESCH, G.W. (1978) Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl. Environ. Microbiol.* **36:** 731-738.

JANDA, J.M. and DUFFEY, P.S. (1988) Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infections disease spectrum. *Rev. Infect. Dis.* **10:** 980-997.

JOSEPH, S.W., DAILY O.P., HUNT, W.S., SEIDLER, R.J., ALLEN, DA.A. and COLWELL, R.R. (1979) *Aeromonas* primary wound infection of a diver in polluted waters. *J. Clin. Microbiol.* **10:** 46-49.

JOSEPH, S.W., JANDA, M and CARNAHAN, A. (1988) Isolation, enumeration and identification of *Aeromonas* spp. *J. Food Saf.* **9:** 23-35.

KAPER, J., SEIDLER, R.J., LOCKMAN, H and COLWELL, R.R. (1979) Medium for the presumptive identification of *Aeromonas hydrophila* and *Enterobacteriaceae*. *Appl. Environ. Microbiol.* **38:** 1023-1026.

KAPER, J.B., LOCKMAN, H and COLWELL, R.R. (1981) *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. *J. Appl. Bacteriol.* **50:** 359-377.

KIELWEIN, G. (1971) Pseudomonaden und Aeromonaden in Trinkmilch: ihr Nachweiss und ihre Bewertung. *Arch. Lebensmittelhyg.* **1:** 15-19.

LEVIN, M.A. and CABELLI, V.J. (1972) Membrane filter technique for enumeration of *Pseudomonas aeruginosa*. *Appl. Microbiol.* **24:** 864-870.

MASCHER, F., REINTHALER, F.F., SIXI, W., SCHUHMANN, G. and ENAYAT, U. (1989) *Aeromonas* spp. aus Trinkwasser und Stuhlproben in Südindien: Isolierung, Charakterisierung und Toxinnachweiss. *Mitt. österr. Ges. Tropenmed. Parasitol.* **11:** 189-196.

McKOY, R.H. and PILCHER, K.S. (1974) Peptone beef extract glycogen agar, a selective and differential *Aeromonas* medium. *J. Fish. Res. Board Can.* **31:** 1553-1555.

MYERS, B.B., MARSHALL, R.T., EDMONDSON, J.E. and STRINGER, W.C. (1982) Isolation of pectinolytic *Aeromonas hydrophila* and *Yersinia enterocolitica* from vacuum-packaged pork. *J. Food. Protect.* **45:** 33-37.

NEILSON, A.H. (1978) The occurrence of Aeromonads in activated sludge: isolation of *Aeromonas sobria* and its possible confusion with *Escherichia coli*. *J. Appl. Bacteriol.* **44:** 259-264.

PALUMBO, S.A., MAXINO, F., WILLIAMS, A.E., BUCHANAN, R.L. and THAYER, D.W. (1985) Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol.* **50:** 1027-1030.

POPOFF, M. and VERON, M. (1976) A taxonomic study of the *Aeromonas hydrophila-Aeromonas punctata* group. *J. Gen. Microbiol.* **94:** 11-22.

POPOFF, M.Y., COYNAULT, C., KIREDJAN, M. and LEMELIN, M. (1981) Polynucleotide sequence relatedness among motile *Aeromonas* species. *Curr. Microbiol.* **5:** 109-114.

POPOFF, M. (1984) Genus III *Aeromonas* Kluyver and Van Niel 1936, 398. In: *Bergey's Manual of Systematic Bacteriology,* N.R. Krieg and J.G. Holt (eds.), pp. 545-548. Williams & Wilkins, Baltimore-London.

RIPPEY, S.R. and CABELLI, V.J. (1979) Membrane filter procedure for enumeration of *Aeromonas hydrophila* in fresh waters. *Appl. Environ. Microbiol.* **38:** 108-113.

RIPPEY, S.R. and CABELLI, V.J. (1980) Occurrence of *Aeromonas hydrophila* in limnetic environments: relationship of the organism to trophic state. *Microbiol. Ecol.* **6:** 45-54.

RIPPEY, S.R. and CABELLI, V.J. (1985) Growth characteristics of *Aeromonas hydrophila* in limnetic waters of varying trophic state. *Arch. Hydrobiol.* **104:** 311-319.

RIPPEY, S.R. and CABELLI, V.J. (1989) Use of the thermotolerant *Aeromonas* group for the trophic state classification of freshwaters. *Wat. Res.* **23:** 1107-1114.

SEIDLER, R.J., ALLEN, D.A., LOCKMAN, H., COLWELL, R.R., JOSEPH, S.W. and DAILY, O.P. (1980) Isolation, enumeration and characterization of *Aeromonas* from polluted waters encountered in diving operations. *Appl. Environ. Microbiol.* **39:** 1010-1018.

SCHUBERT, R.H.W. (1967) Das Vorkommen der Aeromonaden in Oberirdischen Gewässern. *Arch. hyg. Bakt.* **150:** 687-708.

SCHUBERT, R. (1976) The detection of Aeromonads of the "Hydrophila-Punctata-Group" within the hygienic control of drinking water. *Zbl. Bakt. Hyg.* B. **187:** 1-17.

SHOTTS, E.B. and RIMLER, R. (1973) Medium for the isolation of *Aeromonas hydrophila*. *Appl. Microbiol.* **26:** 550-553.

STELMA, G.N., JOHNSON, C.H. and SPAULDING, P. (1986) Evidence for the direct involvement of B hemolysin in *Aeromonas hydrophila* enteropathogenicity. *Curr. Microbiol.* **14:** 71-77.

TORANZO, A.E., SANTOS, Y., NIETO, T.P. and BARJA, J.L. (1986) Evaluation of different assay systems for identification of environmental *Aeromonas* strains. *Appl. Environ. Microbiol.* **51:** 652-656.

TURNBULL, P.C.B., LEE, J.V., MILIOTIS, M.D., VAN DER WALLE, S., KOORNHOFF, H.J., JEFFREY, L. and BRYANT, T.N. (1984) Enterotoxin production in relation to taxonomic grouping and source of isolation of *Aeromonas* species. *J. Clin. Microbiol.* **19:** 175-180.

VAN DER KOOIJ, D., VISSER, A. and HIJNEN, W. (1980) Growth of *Aeromonas hydrophila* at low concentrations of substrates added to tap water. *Appl. Environ. Microbiol.* **39:** 1198-1204.

VAN DER KOOIJ, D. (1988) Properties of Aeromonads and their occurrence and hygienic significance in drinking water. *Zbl. Bakt. Hyg.* B. **187:** 1-17.

WILLIAMS. L.A. and LaROCK, P.A. (1985) Temporal occurrence of *Vibrio* species and *Aeromonas hydrophila* in estuarine sediments. *Appl. Microbiol.* **50:** 1490-1495.

YU, P.K.W. (1985) Appendix B: Media and Reagents. In: *Laboratory Procedures in Clinical Microbiology,* J.A. Washington (ed.), pp. 769-829. Springer-Verlag, New York.

YU, P.K.W. and WASHINGTON, J.A. (1985) Identification of aerobic and facultatively anaerobic bacteria. In: *Laboratory Procedures in Clinical Microbiology,* J.A. Washington (ed.), pp. 131-250. Springer-Verlag, New York.

WATSON, I.M., ROBINSON, J.O., BURKE, V. and GRACEY, M. (1985) Invasiveness of *Aeromonas* spp. in relation to byotipe, virulence factors and clinical features. *J. Clin. Microbiol.* **22:** 48-51.

Campylobacter

AHO, M., KURKI, M., RAUTELIN, H. and KOSUNEN, X. (1989) Waterborne outbreak of *campylobacter* enteritis after outdoors infantry drill in Utti, Finland. *Epidem. Infect.* **103:** 133-141.

ARIMI, S.M., FRICKER, C.R. and PARK, R.W.A. (1988) Occurrence of `thermophilic' campylobacters in sewage and their removal by treatment processes. *Epidemiol. Infect.* **101:** 279-286.

BENJAMIN, J., LEAPER, S.M., OWEN, R.J. and SKIRROW, M.B. (1983) *Current Microbiol.* **8:** 231-238.

BLASER, M.J., BERKOWITZ, I.D., LaFORCE, F.M., CRAVENS, J., RELLER, L.B. and WANG, L.L. (1979) *Campylobacter* enteritis: clinical and epidemiological features. *Ann. Intern. Med.* **91:** 179-185.

BLASER, M.J., HARDESTY, H.L., POWERS, B. and WANG, W.L. (1980) Survival of *Campylobacter foetus* subsp. *jejuni* in biological milieux. *J. Clin. Microbiol.* **11:** 309-313.

BLASER, M.J., TAYLOR, D.N. and FELDMAN, R.A. (1983) Epidemiology of *Campylobacter jejuni* infections. *Epidemiol. Rev.* **5:** 157-176.

BOLTON, F.J. and ROBERTSON, L. (1982a) A selective medium for isolating *Campylobacter jejuni/coli*. *J. Clin. Pathol.* **35:** 462-467.

BOLTON, F.J., HINCHLIFFE, D.M., COATES, D. and ROBERTSON, L. (1982b) A most probable number method for estimating small numbers of campylobacters in water. *J. Hyg.* **89:** 185-190.

BOLTON, F.J., COATES, D., HINCHLIFFE, P.M. and ROBERTSON, L. (1983a) Comparison of selective media for isolation of *Campylobacter jejuni/coli*. *J. Clin. Pathol.* **36:** 78-83.

BOLTON, F.J. and COATES, D. (1983b) Development of a blood-free *Campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplements to facilitate aerotolerance.

BOLTON, F.J., HUTCHINSON, D.N. and COATES, D. (1984) Blood-free selective medium for isolation of *Campylobacter jejuni* from faeces. *J. Clin. Microbiol.* **19:** 169-171.

BOLTON, F.J., COATES, D., HUTCHINSON, D.N. and GODFREE, A.F. (1987) A study of thermophilic campylobacters in a river system. *J. Appl. Bacteriol.* **62:** 167-176.

BOOSINGER, T.R., BLEVINS, W.T., HERON, J.V. and SUNTER, J.L. (1990) Plasmids profiles of six species of *Campylobacter* from human beings, swine, and sheep. *Amer. J. Vet. Res.* **51:** 718-722.

BROKOVIC, V., KALENIC, A. and VODOPIJA, I. (1983) Outbreak of waterborne enteritis in a kindergarten. In: *Campylobacter* II, Pearson A.D., Skirrow M.B., Rowe B., Davies J.R. and Jones D.M., p.147. Public Health Laboratory Service, London. Butzler J.P. and Skirrow M.B. (1979) *Campylobacter* enteritis. *Clin. in Gastroent.* **8:** 737-765.

BUTLER, R.C., LUND, V. and CARLSON, D.E. (1987) Susceptibility of *Campylobacter jejuni* and *Yersinia enterocolitica* to UV radiation. *Appl. Environ. Microbiol.* **53:** 375-378.

CARTE, A.M., PACHA, R.E., CLARK, G.W. and WILLIAMS, E.A. (1987) Seasonal occurrence of *Campylobacter* spp. in surface waters and their correlation with standard indicator bacteria. *Appl. Environ. Microbiol.* **53:** 523-526.

FRICKER, C.R., GIRDWOOD, R.W.A. and MUNRO, D. (1983) A comparison of procedures for the isolation of campylobacters from seagull faeces. *J. Hyg.* **91:** 445-450.

FRICKER, C.R. (1987) The isolation of salmonellas and campylobacters: a review. *J. Appl. Bacteriol.* **63:** 99-117.

FRICKER, C.R. and PARK, R.W.A. (1989) A two-year study of the distribution of `thermophilic' campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *J. Appl. Bacteriol.* **66:** 477-490.

GEORGE, H.A., HOFFMAN, P.S., SMIBERT, R.M. and KRIEG, N.R. (1978) Improved media for growth and aerotolerance of *Campylobacter foetus*. *J. Clin. Microbiol.* **8:** 36-41.

GONDROSEN, B., MELBY, K., GREGUSSON, S and DAHL, O.P. (1985) A waterborne outbreak of *campylobacter* enteritis in the sub-Artic region of Norway. In: *Campylobacter* III, A.D. Pearson, M.B. Skirrow, H. Lior and B. Rowe (eds.), pp. 277, Public Health Laboratory Service, London.

HOFF, J.C. and GELDREICH, E.E. (1982) Adequacy of disinfection for control of newly recognized waterborne pathogens. *Proc. AWWA Water Qual. Tech. Conf.* The American Water Works Assoc.

HOFFMAN, P.S., GEORGE, H.A., KRIEG, N.R. and SMIBERT, R.M. (1979) Studies of the microaerophilic nature of *Campylobacter foetus* subsp. *jejuni*. II Role of exogenous superoxide anions and hydrogen peroxide. *Can. J. Microbiol.* **25:** 8-16.

HOLLER, C. (1988) Long-term study of occurrence, distribution and reduction of *Campylobacter* spp. in the sewage system and wastewater treatment plant of a big town. *Wat. Sci. Tech.* **20:** 529-531.

HUMPHREY, T.J. and CRUICKSHANK, J.G. (1985) Antibiotic and deoxycholate resistance in *Campylobacter jejuni* following freezing or heating. *J. Appl. Bacteriol.* **59:** 65-71.

HUMPHREY, T.J. (1989) An appraisal of the efficacy of pre-enrichment for the isolation of *Campylobacter jejuni* from water and food. *J. Appl. Bacteriol.* **66:** 119-126.

JONES, D.M., ABBOTT, J.D., PAINTER, M.J. and SUTCLIFFE, E.M. (1984) A comparison of biotypes and serotypes of *Campylobacter* sp. isolated from patients with enteritis and from animal and environmental sources.
J. Infect. **9:** 51-58.

JONES, F. and WATKINS, J. (1985) The water cycle as a source of pathogens. *J. Appl. Bacteriol. Symp Supp.* **59:** 27-36.

JONES, K., BETAIEB, M., and TELFORD, D.R. (1990a) Correlation between environmental monitoring of thermophilic campylobacters in sewage effluent and the incidence of *Campylobacter* infection in the community. *J. Appl. Bacteriol.* **69:** 235-240.

JONES, K., BETAIEB, M. and TELFORD, D.R. (1990b) Seasonal variation of thermophilic campylobacters in sewage sludge. *J. Appl. Bacteriol.* **69:** 185-189.

JONES, K., BETAIEB, M. and TELFORD, D.R. (1990c) Thermophilic campylobacters in surface waters around Lancaster, UK: negative correlation with *campylobacter* infections in the community. *J. Appl. Bacteriol.* **69:** 758-764.

KNILL, M., SUCKLING, W.G. and PEARSON, A.D. (1977)1 Environmental isolation of heat-tolerant *Campylobacter* in the Southampton area. Lancet **ii:** 1002-1003.

KADAR, M. (1990) Estimation of *Salmonella* and thermotolerant *Campylobacter* counts in Danube water at Budapest. *Abst. Int. Symp. on Health-Related Water Microbiology,* IAWPR, pp. 26, Tübingen.

KARMALI, M.A. and FLEMING, P.C. (1979) *Campylobacter* enteritis. *Can. J. Microbiol.* **120:** 1525-1532.

LARIVIERE, L.A., GAUDREAU, Ch.L. and TURGEON, F.F. (1986) Susceptibility of clinical isolates of *Campylobacter jejuni* to twenty-five antimicrobial agents. *J. Antimicrob. Chemot.* **18:** 681-685.

LAURIA, A.L. and HOFER, E. (1989) Ocorrencia de *Campylobacter* termofilico em diferentes pontos de una estaçao de tratamento de esgostos na cidade do Rio de Janeiro, *Rev. Microbiol, Sap. Paulo,* **20:** 303-308.

MARTIKAINEN, P.J., KORHONEN, L.K. and KOSUNEN, T.U. (1990) Occurrence of thermophilic campylobacters in rural and urban surface waters in Central Finland. *Wat. res.* **24:** 91-96.

MATHEWSON, J.J., KESWICK, B.H. and DUPONT, H.L. (1983) Evaluation of filters for recovery of *Campylobacter jejuni* from water. *Appl. Environ. Microbiol.* **46:** 985-987.

MENTZING, L.O. (1981) Waterborne outbreaks of *campylobacter* enteritis in central Sweden. *Lancet* **ii:** 352-354.

MERINO, F.J., AGULLA, A., VILLASANTE, P., SAZ, J.V., DIAZ, A. and VELASCO, A.C. (1986) Diagnostico rapido de diarrea por *Campylobacter* sp. mediante examen en fresco y tincion de Gram. *Rev. Esp. Microbiol. Clin.* **1:** 113-114.

NEWELL, D.G. (1982) *Campylobacter*, *Epidemiology, Pathogenesis and Biochemistry,* p.308. MTP Press Ltd., Lancaster.

OWEN, R.J. and HERNANDEZ, J. (1990) Occurrence of plasmids in *"Campylobacter upsaliensis"* (catalase negative or weak group) from geographically diverse patients with gastroenteritis or bacteraemia. *Eur. J. Epidemiol.* **6:** 111-117.

PALMER, S.R., GULLY, P.R., WHITE, J.M., PEARSON, A.D., SUCKLING, W.G., JONES, D.M., RAWES, J.C.L. and PENNER J.L. (1983) Waterborne outbreak of *Campylobacter* gastroenteritis. *Lancet* **1:** 287-290.

PARK, C.H., HIXON, D.L., POLEHEMUS, A.S., FERGUSON, C.B., HALL, S.L., RISHEIM, C.C., BARRIE, C. (1983) A rapid diagnosis of *Campylobacter* enteritis by direct smear examination. *Amer. J. Clin. Pathol.* **80:** 388-389.

PEARSON, A.D., LIOR, H., HOOD, A.M. and HAWTIN, P. (1985) Longitudinal study of the occurrence, biotype and serogroup of *Campylobacter jejuni* and *C. coli* isolations from environmental sources in southern England between 1977 and 1985. In: *Campylobacter* III. Pearson A.D., Skirrow M.B., Lior H. and Rowe P. (eds.), pp. 281-282. Public Health Laboratory Service, London.

ROBINSON, D.A. (1981) Infective dose of *Campylobacter jejuni* in milk. *British. Med. J.* **282:** 1584.

ROGOL, M., SECHTER, I.M., FALK, H., SHTARK, Y. ALFI, S., GREENBERG, Z. and MIZRACHI, R. (1983) Waterborne outbreak of *Campylobacter* enteritis. *Eur. J. Clin. Microbiol.* **2:** 588-590.

ROLLINS, D.M. and COLWELL, R.R. (1986) Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Appl. Environ. Microbiol.* **52:** 531-538.

ROSEF, O., GONDROSEN, B. KAPPERUD, G. and UNDERDAL, B. (1983) Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl. Environ. Microbiol.* **46:** 855-859.

ROSEF, O., KAPPERUD, G. and SKJERVE, E. (1987) Comparison of media and filtration procedures for qualitative recovery of thermotolerant *Campylobacter* spp. from naturally contaminated surface water. *Int. J. Fd. Microbiol.* **5:** 29-39.

SANDSTEDT, K. and URSING, J. (1986) *Campylobacter upsaliensis,* a new species, formerly the CNW group. *Abst. XIV Int. Cong. Microbiol.* pp. 61, IUMS, Manchester.

SKIRROW, M.B. (1977) *Campylobacter* enteritis- a `new' disease. *British Med. J.* **ii:** 9-11.

SKIRROW, M.B. (1987) A demographic survey of *Campylobacter, Salmonella* and *Shigella* infections in England. *Epidemiol. and Infect.* **99:** 647-657.

SMIBERT, R.M. (1984) Genus *Campylobacter* Sebald and Veron. In: *Bergey's Manual of Systematic Bacteriology,* N.R. Krieg and J.G. Holt (eds.), pp. 111-118. Williams & Wilkins, Baltimore-London.

STELZER, W., MOCHMAN, H., RICHTER, U. and DOBBERKAU, H.J. (1988) Characterization of *Campylobacter jejuni* and *Campylobacter coli* isolated from waste water. *Zbl. Bakt. Hyg.* **269:** 188-196.

STELZER, W., MOCHMAN, H. RICHTER, U. and DOBBERKAU, H.J. (1989) A study of *Campylobacter jejuni* and *Campylobacter coli* in a river system. *Zbl. Hyg.* **189:** 20-28.

STELZER, W. (1990) Detection and spread of *Campylobacter* in water. *Abst. Int. Symp. on Health-Related Water Microbiology,* IAWPRC, pp. 27, Tübingen.

SVEDHEM, A. and KAIJSER, B. (1980) *Campylobacter foetus* spp. *jejuni*: a common cause of diarrhoea in Sweden. *J. Infect. Dis.* **142:** 353-359.

TAYLOR, D.N., McDERMOTT, K.T., LITTLE, J.R., WELLS, J.G. and BLASER, M.J. (1983) *Campylobacter* enteritis from untreated water in rocky mountains. *Ann. Int. Med.* **99:** 38-40.

WALKER, R.I., CALDWELL, M.B., LEE, E.C. *et al.* (1986) Pathophysiology of *Campylobacter* enteritis. *Microbiol. Rev.* **50:** 81-94.

7. APPENDIX: PHOTOGRAPHS

Photo 1: Effluent discharge point at Puzol sampling area

Photo 2: Effluent discharge point at Puig sampling area

Photo 3: Effluent discharge point at Puebla de Farnals area

Photo 4: Effluent discharge point at Port Saplaya area

Photo 5: Effluent discharge point at Malvarrosa area

Photo 6: Saler sampling area (sites 1 and 2)

Photo 7: Perello sampling area (sites 1 and 2)

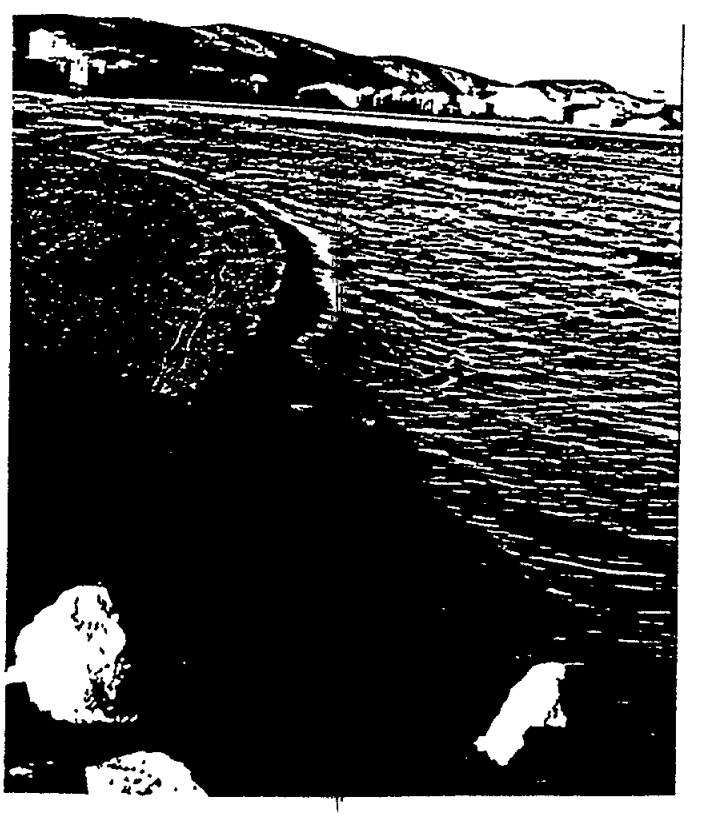

Photo 8: Site 2 at Cullera sampling area

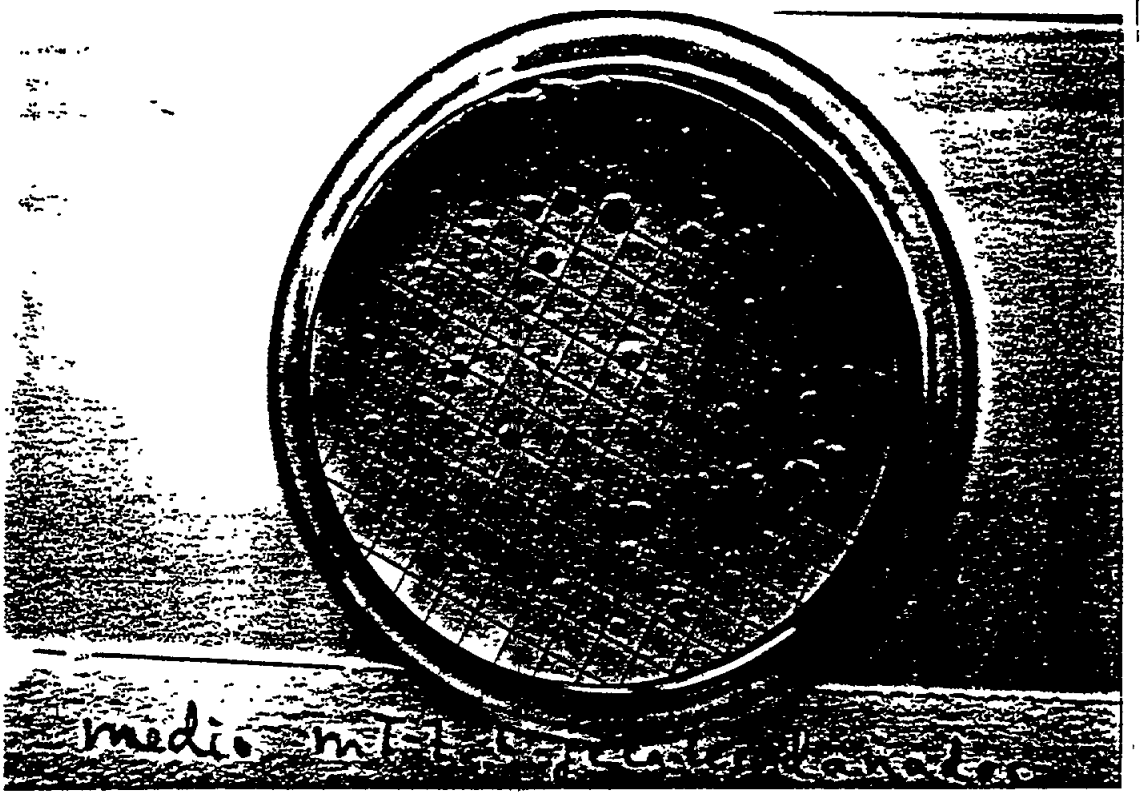

Photo 9: Colonies of faecal coliforms (yellow colonies)
on m-T7 agar

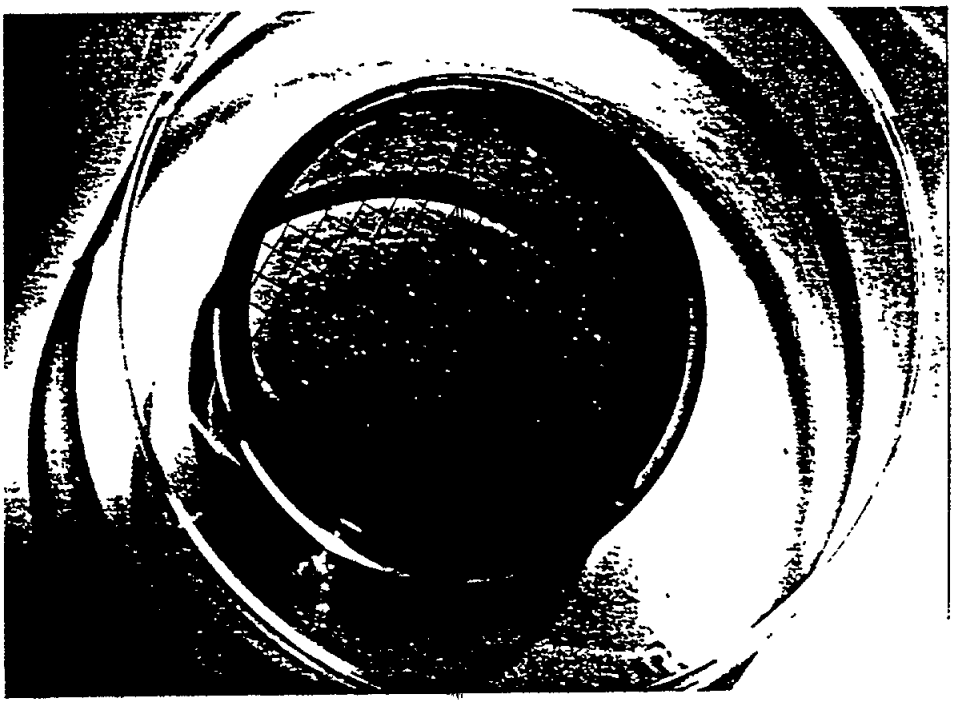

Photo 10: Colonies of motile Aeromonads (yellow colonies)
on mADA/0129 agar

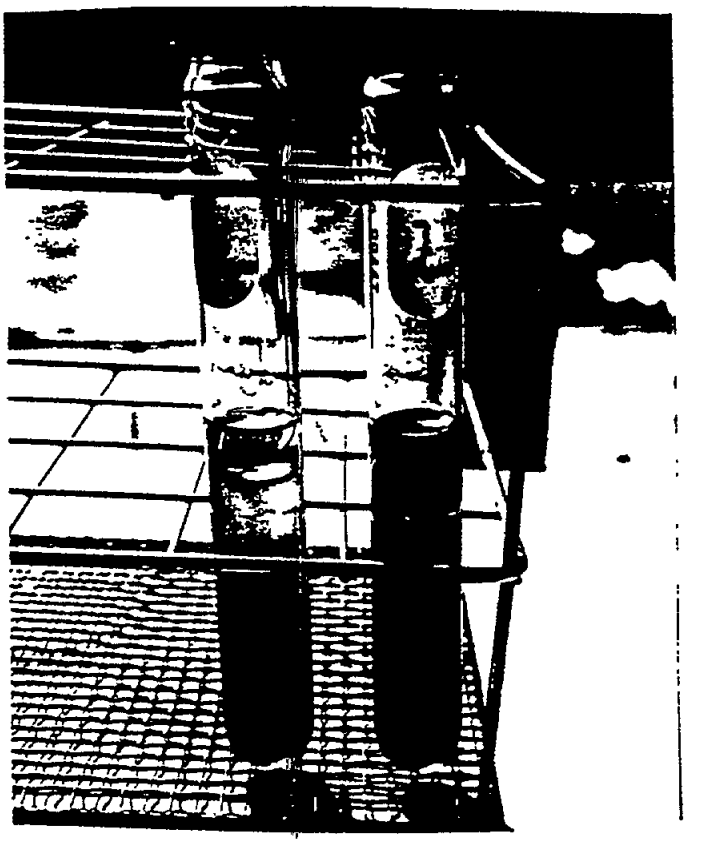

Photo 11: Identification of motile Aeromonads with the AH medium (tube 1 with Kovac's reagent)

Photo 12: Identification of motile *Aeromonas* species with esculine agar and, acid and gas formation from glucose, from left to right: *A. caviae*, *A. sobria*, *A. hydrophila* and *Aeromonas* spp.

Photo 13: Growth of *Campylobacter* colonies on Preston agar

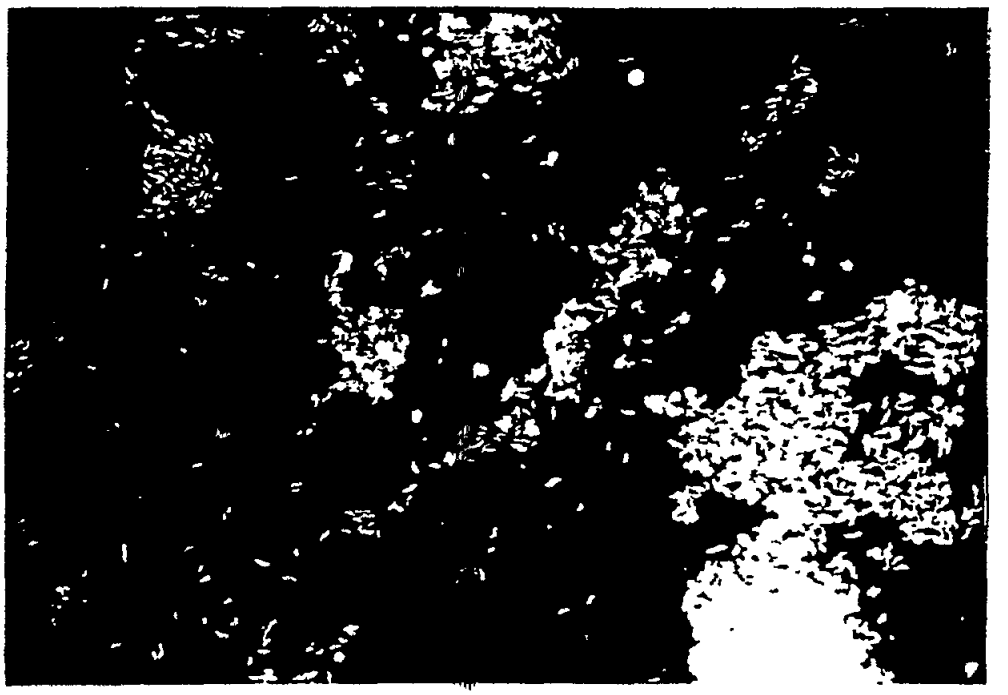

Photo 14: Observation of campylobacters stained with acridine orange (fluorescence microscopy, 1,000X)

Photo 15: Catalase test

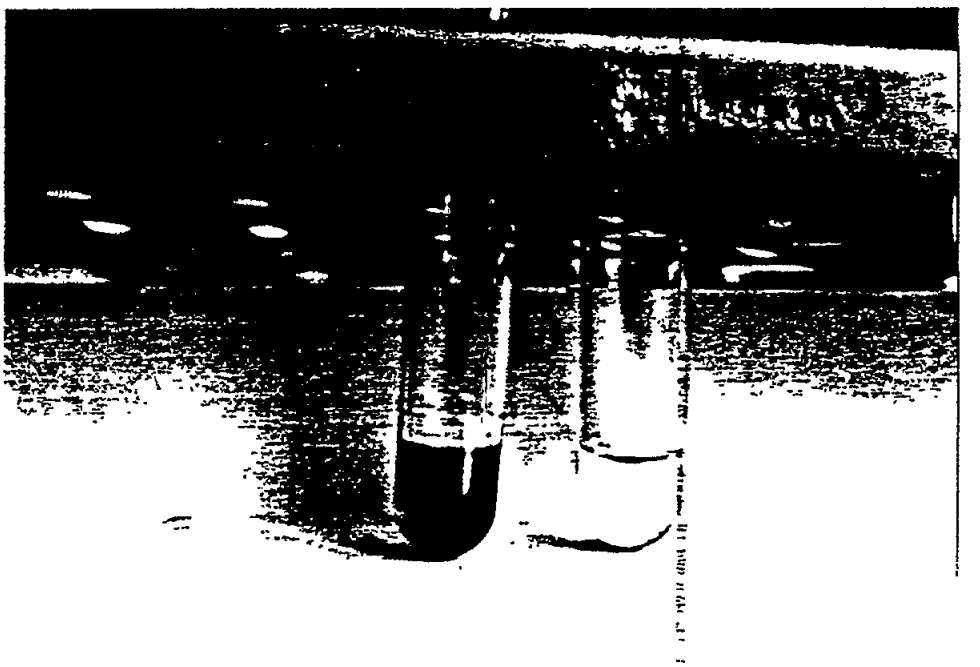

Photo 16: Hippurate hydrolysis

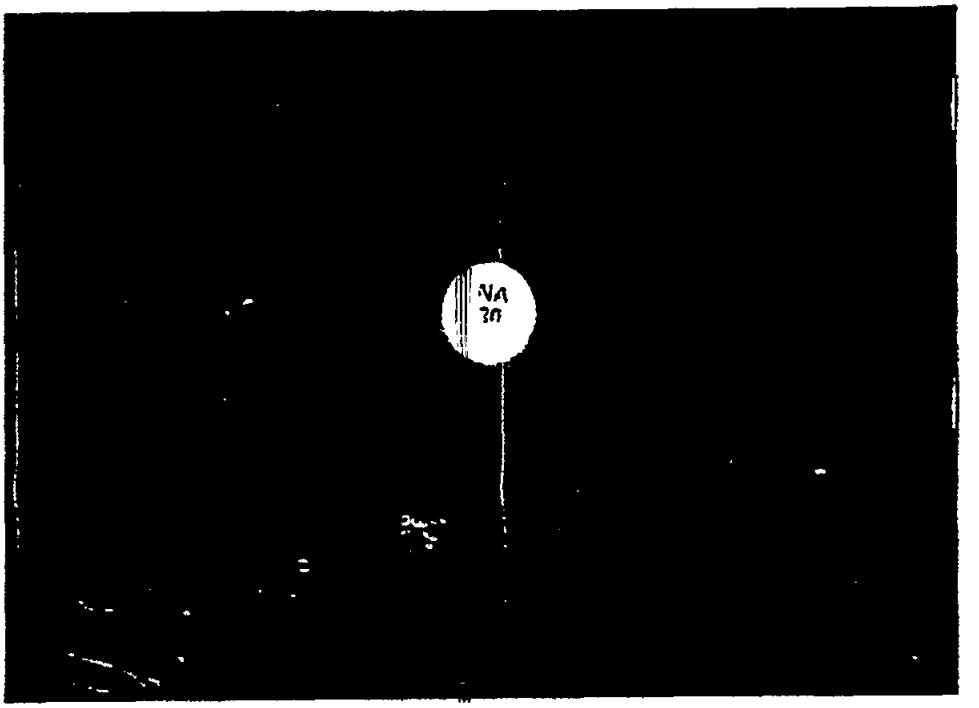

Photo 17: Sensitivity to nalidixic acid (30 µg disk)

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73. PNUE/FAO: Rapports finaux sur les projets de recherche traitant des effets de polluants sur les communautés et les organismes marins. MAP Technical Reports Series No. 73. UNEP, Athens, 1993 (186 pages) (anglais et français).

74. PNUE/FIS: Rapport de l'Atelier de formation sur les aspects de la documentation marine en Méditerranée. MAP Technical Reports Series No. 74. UNEP, Athens, 1993 (38 pages) (anglais seulement).

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