EP

United Nations
Environment
Programme

UNEP(DEPI)/MED WG. 316/Inf. 5
June 2007

ENGLISH

MEDITERRANEAN ACTION PLAN
MED POL

Meeting of MED POL National Coordinators

Hammamet (Tunisia), 25-28 June 2007

ASSESSMENT OF THE STATE OF MICROBIAL POLLUTION OF THE MEDITERRANEAN SEA

In cooperation with

WHO

UNEP/MAP
Athens, May 2007

Table of Contents

Page no.

Page no.

Introduction

One of the main causes of the deterioration of the state of the Mediterranean Sea was identified in late 60s as the considerable amount of largely-uncontrolled coastal discharges of untreated or partially-treated municipal and industrial wastes. By the early 1970s, a substantial amount of data on various aspects of Mediterranean pollution had already been recorded. It became increasingly apparent that the situation created an equal, if not greater, threat to human health, both through bathing in polluted coastal seawater and through consumption of microbiologically or chemically contaminated seafood. Early studies on those aspects of pollution having a direct or indirect bearing on human health consisted, in the main, in the measurement of concentrations of various bacteria (mainly the orthodox indicators of sewage pollution) in coastal bathing and shellfish areas, and of a number of organic and inorganic chemicals in various species of seafood. The situation attracted the attention of the major United Nations Agencies, which immediately recognised the need for a properly-balanced region-wide programme in order to reach a reasonable-accurate estimate on the actual state of pollution of the Mediterranean Sea.

In February 1976, in Barcelona, Mediterranean States adopted and signed the Convention for the Protection of the Mediterranean Sea against Pollution, along with two protocols dealing respectively with pollution from dumping by ships and aircraft, and cooperation in pollution emergencies (UNEP, 1978).

The Protocol for the Protection of the Mediterranean Sea against pollution from Land-based Sources was adopted and signed in Athens on 17 May 1980. Under the terms of Article 7.1 of the Protocol (UNEP, 1980), Contracting Parties are bound to progressively formulate and adopt, in cooperation with the competent international organizations, common guidelines and, as appropriate, standards or criteria dealing in particular with a number of aspects, including the quality of seawater used for specific purposes that is necessary for the protection of human health, living resources and ecosystems. Following agreement on a number of issues, including consolidation of Annexes I and II into one Annex, which also includes a list of terrestrial activities linked with marine pollution, amendments to the 1980 Land-based Sources Protocol were formally adopted and signed during a Conference of Plenipotentiaries convened by UNEP in Syracuse from 6 to 7 March 1996, the new title of the Protocol becoming "Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources and Activities" (UNEP, 1996).

The amendments made to the 1976 Barcelona Convention on the Protection of the Mediterranean Sea against Pollution (UNEP, 1995a, 1995b) and to the 1980 Athens Protocol for the Protection of the Mediterranean sea against Pollution form Land-based Sources in no way affect the importance accorded to the prevention and control of microbiological pollution of the Mediterranean Sea. In general terms, Article 8 of the revised version of the Convention further strengthens the importance accorded to land-based pollution, while in the new version of the Protocol, Article 7.1 remains unchanged and Pathogenic microorgnanisms retain their place in the new Annex I. Similarly, the revisions do not affect the general procedures for the preparation, development and adoption of any individual or joint measures designed to achieve this purpose.

The problem of microbiological pollution in the marine environment of the region was specifically recognised by Mediterranean states when adopting the MED POL Programme in 1975. One particular pilot project within this component of the Action Plan (FAO/UNESCO/IOC/WHO/WMO/IAEA/UNEP, 1983) entitled "Coastal Water Quality Control" (MED POL vii) entailed the regular monitoring by designated Mediterranean national institutions of coastal recreational waters, shellfish-growing water and shellfish flesh, the main parameters being microbiological. Thirty institutions from fourteen Mediterranean countries participated in the pilot project, which was coordinated by the World Health

Organization. During the course of the pilot project, desirable environmental quality criteria for both recreational and shellfish-growing waters were elaborated by participants, with a view to their eventual proposal for adoption by Mediterranean Governments.

The Second Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols, held in Cannes from 2 to 7 March 1981 (UNEP, 1981), approved the second phase of the programme, now termed the Long-term Programme of Pollution Monitoring and Research in the Mediterranean sea (MED POL Phase II). Originally designed to cover the period 1981-1990, the programme was later extended until the end of 1995. The monitoring component of the programme (UNEP, 1983), included microbiological monitoring of recreational and shellfish waters within the framework of national marine pollution monitoring programmes to be upgraded or established.

Previous assessment of microbiological pollution of the Mediterranean Sea

In view of the fact that interim microbiological criteria for recreational and shellfish waters had already been prepared during the first phase of the MED POL Programme, these were proposed to the Governments of the region within the framework of an assessment on the current state of microbial pollution of the Mediterranean Sea (UNEP/WHO, 1985), which was prepared by WHO mainly on the basis of monitoring data from the MED POL vii pilot project. The Fourth Ordinary Meeting of the Contracting Parties to the Convention and Protocols, held in Genoa in 1985 decided to postpone the issue of shellfish waters and shellfish to a later date. Insofar as the recommendations regarding recreational waters were concerned, these were approved only in part, Contracting Parties adopting joint interim criteria for bathing waters based on maximum acceptable concentrations of only one indicator organism (faecal coliforms), instead of the two (faecal coliforms and faecal streptococci) proposed. Details of the proposals and the interim criteria for recreational waters adopted are contained in Part 4 of this document.

At this Fifth Ordinary Meeting, held in Athens from 8 to 11 September 1987, (UNEP, 1987), Contracting Parties adopted environmental quality criteria for shellfish waters, proposed on the basis of a revised assessment (UNEP/WHO, 1987) prepared by WHO on the recommendations of a meeting of experts convened by that Organization earlier that year (UNEP/WHO, 1987), which had the task of preparing alternative proposals to those submitted in 1985. The criteria recommended, and eventually adopted, were limited to shellfish waters, and were identical with the relevant EC Directive on the subject (EC, 1979). In this context, it was understood that the scope of the resolution in question, the operative parts of which are given in Part 4 of this document, was only designed to cater for acceptability of marine areas for shellfish growing and harvesting, and did not in any way intrude on acceptability of the shellfish for human consumption, which aspect would continue to be handled by appropriate public health or related legislation in the various countries (WHO, 1989).

The first ad hoc assessment of the state of pollution of the Mediterranean Sea by pathogenic microorganisms was prepared by WHO in 1991 (UNEP/WHO, 1991) and submitted to the seventh ordinary meeting of the Contracting Parties to the Barcelona Convention and Protocols (UNEP, 1991). Except for a brief review of the situation regarding temporal trends in concentrations of bacterial indicator organisms in coastal (mainly recreational) waters on the basis of an interim review of the MED POL Phase II monitoring data (UNEP, 1989), and data from Mediterranean States contained in annual EC reports on bathing waters, the document concentrated on pathogenic microorganisms recorded in the Mediterranean, their source, dispersal and fate, and on microbiological/epidemiological studies conducted to date on the correlation between coastal water quality and health effects on exposed population groups. A number of recommendations on data acquisition through monitoring and research were made. It was not, however, recommended that, at that particular state in time, any formal action to be taken to amend the current interim microbiological criterion for acceptability of bathing waters, even on a further interim basis, as the global situation regarding the validity of the several bacterial indicators in current use was in a state of flux.

In 1996 an "Assessment of the state of Microbiological pollution of the Mediterranean Sea" was prepared by the World Health Organization and was issued jointly by the Untied Nations Environment Programme and in particular for the MED POL Programme of the Mediterranean Action Plan. That document attempted to consolidate and update all previous information on the state of microbiological pollution of the Mediterranean sea with particular reference to coastal recreational and shellfish areas through the inclusion of monitoring and research data, drawn from national MED POL monitoring programmes, MED POL research projects, EC annual reports on bathing waters, and other national and international sources. Wherever appropriate, relevant data from previous documents were included, either in the original or in an abridged form, as appropriate, to make the document more self-contained, and provide a better view of temporal trends. An overview of the current situation, together with recommendations for possible action was also included (UNEP/MAP/WHO, 1996).

Scope of the present document

In view of the relative importance of microbiological pollution of coastal areas and according to the adoption of the workplan by the National MED POL Coordinators for the biennium 2006-2007, the present document can be seen as a tool that provides updated information on microbiological pollution in the Mediterranean Sea and in addition compares the results of the 1996 report with the data from the 1996-2006 decade. It should also be taken into consideration, that since that time several changes occurred. For example, more countries joined the European Union, being subjects to more stringent legislation, followed by a number of non-European countries, which decided to adopt or to follow the European directives. Moreover, a considerable amount of monitoring data mainly concerning recreational waters were generated and available for interpretation, related to compliance with the relevant legislation.

But it should also be noted that in the last years, due to recent advances in the field of epidemiological studies correlating bathing water quality and health effects, the World Health Organization published in 2003 the "Guidelines for safe recreational water environments" and the European Commission launched in 2006 the updated Directive "concerning the management of bathing water quality".

The present document was prepared by the World Health Organization for the MED POL Programme of the Mediterranean Action Plan with the assistance of a number of consultants, while the available compliance data were either provided by the MED POL National Coordinators, or were drawn from national MED POL monitoring programmes, or EC annual reports on bathing waters. The assessment is based on the results of compliance monitoring programmes that directly highlight the degree of compliance to the national, Mediterranean or EU legislation.

1. SOURCES OF POLLUTION

In both coastal and freshwaters the point sources of pollution that cause most health concern are those due to domestic sewage discharges. Diffuse outputs and catchments aggregates of such pollution sources are more difficult to predict. Discharge of sewage to coastal and riverine waters exerts a variable polluting effect that is dependent on the quantity and composition of the effluent and on the capacity of the receiving waters to accept that effluent. Thus enclosed, low volume, slowly-flushed water systems will be affected by sewage discharges more readily than will water bodies that are subject to rapid change and recharge (CEC, EPA, WHO; 2000).

Currently, waters used for bathing and shellfish harvesting have particular requirements regarding quality. Methods to differentiate animal from human sources of faecal pollution will assist water resource managers in developing strategies to protect shellfish harvesting areas and recreational waters and thus reduce the public health risk from these waters. A review of methods used to source-track microbial pollution can be found in Pond *et al.,* (2004).

1.1 Pollution derived from sewage effluents

Recreational waters generally contain a mixture of pathogenic and non-pathogenic micro-organisms. These micro-organisms may be derived from sewage effluents, the recreational population using the water (from defecation and/or shedding), livestock (cattle, sheep, etc.), industrial processes, farming activities, domestic animals (such as dogs) and wildlife. In addition, recreational waters may also contain free-living pathogenic micro-organisms (WHO, 2003). These sources can include pathogenic organisms that cause gastrointestinal infections following ingestion or infections of the upper respiratory tract, ears, eyes, nasal cavity and skin. This is further discussed in section 3.

During the fourth ordinary meeting of the Contracting Parties to the Barcelona Convention and Protocols in Genoa in September 1985, Mediterranean States adopted a formal Declaration (subsequently termed the Genoa Declaration) wherein they committed themselves to the achievement of a number of environmental targets during the second decade of operation (1986-1995) of the Mediterranean Action Plan. These targets included the establishment as a matter of priority of sewage treatment plants in all cities around the Mediterranean with more than 100,000 inhabitants, and appropriate outfalls and/or appropriate treatment plants for all towns with more than 10,000 inhabitants (UNEP, 1985).

In 2004 UNEP/MAP published a document containing information on the municipal wastewater treatment plants in Mediterranean coastal cities with more than 10,000 inhabitants (UNEP/MAP/WHO, 2004). The summary of the information is presented in Table 1.

Table 1
Municipal wastewater treatment plants in Mediterranean coastal cities

Total number of countries	19	
Total number of cities	601	
Total number of wastewater treatment plants	665	
Total number of wastewater treatment plants	665	
(1) Cities without a wastewater treatment plant	*138*	*21%*
Cities with a wastewater treatment plant under construction / projected	*40*	*6%*
Cities with a wastewater treatment plant on maintenance /out of operation	*31*	*4%*
Cities with a wastewater treatment plant	*456*	*69%*
Cities with a wastewater treatment plant	456	
(a) Pre-treatment	*9*	*2%*
Primary treatment	*83*	*18%*
Secondary treatment	*249*	*55%*
Tertiary treatment	*68*	*15%*
Unknown treatment	*47*	*10%*
Total number of cities for which population was reported	593	
Total number of cities with more than 100,000 inhabitants	*104*	
Total number of cities with more than 10,000 inhabitants and less than 100,000 inhabitants	*464*	
Total number of cities with less than 10,000 inhabitants (included due to seasonal population)	*25*	
Total number of cities with more than 100,000 inhabitants	104	
Total number of cities with more than 100,000 inhabitants served by a treatment plant	*77*	*74%*
Total number of cities with more than 100,000 inhabitants not served by a treatment plant	*27*	*26%*
Total number of cities with more than 10,000 inhabitants and less than 100,000 inhabitants	489	
Total number of cities with more than 10,000 inhabitants and less than 100,000 inhabitants served by a treatment plant	*332*	*68%*
Total number of cities with more than 10,000 inhabitants and less than 100,000 inhabitants not served by a treatment plant	*157*	*32%*
Total number of "resident" population reported	58,730,024 (for 593 cities)	
Population served by a sewerage network and a treatment plant (included the population that is due to be served)	*52,242,800*	
Total cubic metres of wastewater treated per day (for reported information) Respective population	≈ 6.1 million ≈ 36.7 million	84%
Total cubic metres of untreated wastewater per day (for reported information)* Respective population	≈ 1.15 million ≈ 16.2 million	16%
Total wastewater, cubic metres per capita per day	0.120	

*Note that this quantity is not totally discharged in the aquatic environment.
Source: UNEP/MAP/WHO, 2004.

Data from the study show that, at the time of the submission, in Algeria 38% of the total population was served by wastewater treatment plants; in France 950,000 cubic meters of treated wastewater is disposed to the sea (82% of the total quantity) or in surface waters (12% of the total quantity); in Greece 10% of the wastewater produced is untreated, although it should be noted that untreated sewage is not directly discharged to the marine environment since in most cases raw sewage from households is collected to septic tanks; in Israel there is no discharge of untreated wastewater while treated wastewater is discharged to the sea through submarine outfalls (7%) or is reused (93%), while in Turkey 62% of the population is being reported as having wastewater treatment facilities (19 wastewater treatment plants serve about 3 million habitants) (UNEP/MAP/WHO, 2004).

Regulatory schemes for the microbial quality of recreational water have been largely based on percentage compliance with faecal index organism counts (EC, 1976). Management actions are retrospective and can be deployed only after human exposure to the hazard. In many situations, the risk to health is primarily from human excreta, yet the traditional indices of faecal pollution are also derived from other sources. The response to non-compliance, however, typically concentrates on sewage treatment or outfall management. Beaches are classified as either safe or unsafe, although there is, in fact, a gradient of increasing variety and frequency of health effects with increasing faecal pollution of human and animal origin.

1.2 Pollution derived from the atmosphere

The atmosphere may also serve as a pathway for the entry of pathogenic and other micro-organisms into the coastal marine environment. It has been stated (Brisou, 1976) that winds blowing from the continents towards the sea carry, *inter alia*, bacteria, viruses and parasites, and that rain facilitates the descent of these pollutants into rivers and oceans.

1.3 Pollution derived from bathers

One other possible source of pollution, which affects mainly coastal recreational areas, is bathers themselves. Recreational waters not affected by sewage effluent discharges can be contaminated with enteroviruses, and that the serotype found in the water is likely to be the same one predominating in concomitant human infections (Shuval, 1986). Therefore, bathing waters contaminated by the bathers themselves may at times serve as an effective route of transmission of some viral diseases. This could also apply to other bacterial and fungal infections (Papadakis *et al.,* 1997), and it has been reported that skin counts of certain bacteria and fungi can increase after bathing, even if the water is unpolluted (Papapetropoulou and Sotiracopoulou, 1995)

1.4 Algal blooms

In coastal and estuarine waters, algae range from single-celled forms to seaweeds. Cyanobacteria are organisms with some characteristics of bacteria and some of algae. They are similar in size to the unicellular algae and, unlike other bacteria, contain blue-green or green pigments and are able to perform photosynthesis; thus, they are also termed blue-green algae.

Algal blooms in the sea have occurred throughout history but have increased during recent decades (Anderson, 1989; Smayda, 1989a; Hallegraeff, 1993). In several areas (e.g., the Baltic and North seas, the Adriatic Sea, Japanese coastal waters and the Gulf of Mexico), algal blooms are a recurring phenomenon. The increased frequency of occurrence has accompanied nutrient enrichment of coastal waters on a global scale (Smayda, 1989b).

Blooms of non-toxic phytoplankton species and mass occurrences of macro-algae can affect the amenity value of recreational waters due to reduced transparency, discoloured water and scum formation. Furthermore, bloom degradation can be accompanied by unpleasant odours, resulting in aesthetic problems (WHO, 2003).

Several human diseases have been reported to be associated with species of dinoflagellates, diatoms, nanoflagellates and cyanobacteria that occur in the marine environment (CDC, 1997). The effects of these algae on humans are due to some of their constituents, principally algal toxins. Marine algal toxins become a problem primarily because they may concentrate in shellfish and fish that are subsequently eaten by humans (CDR, 1991; Lehane, 2000), causing syndromes known as paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP) and ciguatera fish poisoning (CFP).

1.5 Eutrophication

Eutrophication can be defined as the process of enrichment of waters with plant nutrients, primarily nitrogen and phosphorus, which stimulates aquatic primary production. Apart from algal blooms (or red tides), its most serious manifestations are algal scum, enhanced benthic algal growth leading at times to massive growth of submerged and floating macrophytes. Sometimes these manifestations are accompanied by, or alternate with, cycles of visible bacterial blooms and fungal development. Eutrophication as a water quality problem differs from pollution-related ones mainly in the increased difficulty in distinguishing the process of eutrophication caused by man from processes and phenomena that may also occur naturally. Although not its main cause, sewage and other forms of water pollution may directly or indirectly enhance or counteract eutrophication. In this context, it has been stated that it has been firmly established that there is a direct correlation between the number of red tide events and the extent of coastal pollution, particularly from sewage and some forms of industrial waste (UNEP/ MAP/WHO, 1996).

2. PATHOGEN SURVIVAL - THE NEW APPROACH TO THE PHENOMENA

A review of the scientific literature on the survival of enteric bacteria in the marine environment reveals a set of attempts, many of them highly empirical in nature, to describe the actual fate of wastewater or enteric bacteria upon exposure to seawater (UNEP/MAP/ WHO, 1996). Only in recent years have these been expanded to include also some molecular aspects of the studied phenomena. A recurring point arising from the data collated is that in addition to actual seawater incubation conditions, previous growth history also has a major influence on subsequent survival in the hostile marine environment. This clearly indicates the existence of an adaptation potential: at least some of the risks inherent in seawater exposure can be handled better if the cells can first mobilize the necessary defense circuits.

The molecular data available to date clearly point at possibly the most significant among such adaptive systems: the rpoS regulon. The sigma factor RpoS (sigmaS) has been described as a general stress response regulator that controls the expression of genes which confer increased resistance to various stresses in some gram-negative bacteria. At least 50 different genes were previously shown to be under rpoS control; they are induced upon a shift to a stationary growth phase, as well as by diverse stresses including salinity and starvation. Not all rpoS-controlled genes were assigned a clear function, but among those that were, many were involved in combating the effects of such stresses. The dominance of this regulatory circuit was observed both in the significant negative effect that rpoS mutations had on *E. coli* survival (Munro *et al.*, 1994, 1995) and in the observation that rpoS-dominated genes accounted for 18 out of 22 shown to be induced by sea water exposure (Rozen *et al.*, 2001).

While it is generally accepted that one of the earlier phenomena observed in enteric bacteria exposed to seawater is the loss of the ability to form colonies on solid media, there is a controversy in regard to the physiological state of the non-culturable cells (Rozen and Belkin, 2001). Bogosian *et al.* (1996, 1998) claim that non-culturable cells are either dead or of no significance as they cannot be resuscitated if experimental procedures are carefully carried out. In contrast, other evidence suggests that they are not only viable but that the pathogenic among them may still be infective (Pommepay *et al.*, 1996). Such an observation has a major significance in view of the worldwide practice of releasing non-disinfected wastewaters into the sea and its potential public health consequences. The seawater-related non-culturable cells controversy is a part of the broader issue of the true meaning of different viability criteria and of the molecular and biochemical mechanisms controlling the shift from colony forming to non- culturable cells and vice versa. These regulatory systems are only partially understood, and probably vary according to the stimuli imposed on the cells (Rozen and Belkin, 2001). Another limitation is that the significance of many of the field experiments is often site-specific, and they tend to ignore previous growth history of the monitored strains.

Thus, mathematical models based on such results cannot replace the need for routine bacterial pollution monitoring. Conversely, laboratory experiments can be accurately designed to test the effects of specific parameters, but cannot simulate or imitate the complexity of the real marine environment. Nevertheless, a true understanding of responses of enteric bacteria to the imposed conditions and their molecular controls can only be obtained in the laboratory. Another front in which basic molecular studies will be able to markedly affect wastewater pollution monitoring practices are the detection methodologies of either pathogenic or indicator micro-organisms. Present approaches are based upon a few simple though highly informative indicator assays. In recent years, several molecular approaches have been proposed that allow fast and sensitive detection of bacteria, viruses and other organisms. Such methods may lead to a more accurate and rapid wastewater pollution detection, including bacteria in the viable but non- culturable state. These methods will provide a broader understanding of the survival of these organisms, thus meeting the challenge of future health management of marine waters. In order to continue to unravel the dominant mechanisms in seawater survival of enteric bacteria, future research will have to combine traditional field and laboratory viability experiments with the molecular insight allowed by genetic approaches. In addition to enhancing our understanding of the basic mechanisms involved in the defences against multiple stress conditions, such studies will surely also allow a more knowledgeable forecast of bacterial decay rates in the sea.

2.1 Survival variation depending on strain

Ideally, all strains of a given indicator organism would experience equal persistence in water; however, some strains may have comparatively extended persistence outside the host, while others may persist very poorly in environmental waters. Assessment of the relative contribution of host species to faecal pollution would be confounded by differential persistence of strains. Faecal coliform decay rates seem to be significantly lower than those of enterococci in freshwater but were not significantly different in saltwater (Anderson *et al.*, 2005). Rates of inactivation of water quality indicators, total coliforms (TC), *Escherichia coli*, enterococci (EC) and F+-specific coliphage were studied (Noble *et al.*, 2004). EC degraded the slowest in the dark with T90s of 115-121 and 144-177 h at 20 and 14 degrees C, respectively. When incubated in sunlight, EC was inactivated significantly more rapidly than either *E. coli* or F+-specific coliphage ($P < 0.001$).

There is particular concern about the survival patterns of *Cryptosporidium parvum* due to a number of epidemics provoked by the consumption of drinking water. The survival of various isolates of *C. parvum* oocysts under a range of environmental pressures including freezing, desiccation, and water treatment processes and in physical environments commonly associated with oocysts such as faeces and various water types was monitored. Oocysts demonstrated longevity in all water types investigated, including seawater, and when in contact with faeces were considered to develop an enhanced impermeability to small molecules which might increase the robustness of the oocysts when exposed to environmental pressures (Robertson *et al.*, 1992).

2.2 Environmental factors affecting the survival of faecal bacteria in marine waters

When enteric bacteria are exposed to seawater they are simultaneously challenged by a combination of stress factors, including pH, temperature, salinity, nutrient availability, light radiation and its associated oxidative stress. It is claimed that the latter two may probably be the most significant components of this hostile combination, though relevant only for shallow waters of coastal areas (UNEP/MAP/WHO, 1996). Salinity itself appears to be less significant (Rozen and Belkin, 2001); indeed, when supplied with sufficient organic nutrients, *E. coli* can grow in seawater almost as well as it does in rich laboratory media and out-compete marine strains. Nevertheless, the lower survival of *E. coli* compared to the bacterial and viral pathogens under laboratory conditions raises concerns because it is a key microbial indicator of faecal contamination.

2.2.1 Light radiation

A study from the UK reports the results of irradiated microcosm experiments using simulated sunlight to investigate the decay of intestinal enterococci in relatively turbid estuarine and coastal waters collected from the Severn Estuary and Bristol Channel, UK. High-turbidity estuarine waters produced a T_{90} value of 39.5 h. Low-turbidity coastal waters produced a much shorter T_{90} value of 6.6 h. In experiments receiving no irradiation, high-turbidity estuarine waters also produced a longer T_{90} of 65.1 h compared with corresponding low-turbidity coastal waters, T_{90} 24.8 h. Irradiated T_{90} values were correlated with salinity, turbidity and suspended solids ($r>0.8$, $p<0.001$). The results suggest that enterococci decay in irradiated experiments with turbidity >200 NTU is similar to decay observed under dark conditions. (Kay *et al.*, 2005).

Gourmelon *et al.* (1997) investigated the effect of visible light on *E. coli* in seawater microcosms. *E. coli* lost its ability to form colonies in marine environments when exposed to artificial continuous visible light. Survival of illuminated bacteria during the stationary phase was drastically reduced in the absence of the sigma factor (RpoS or KatF) that regulates numerous genes induced in this phase. In the stationary phase, double catalase mutants katE katG and mutants defective in the protein Dps (both catalase and Dps are involved in resistance to hydrogen peroxide (H_2O_2)), were more sensitive to light. In the exponential phase, a mutation in oxyR, the regulatory gene of the adaptive response to H_2O_2, increased sensitivity to light, further suggesting that deleterious effects might be associated with H_2O_2 production. However, in the stationary phase, the katE katG dps mutant was considerably more resistant to visible light than the rpoS mutant, suggesting rpoS-dependent protection against deleterious effects other than those related to H_2O_2.

Microcosm studies have been carried out to find out the relative survival of *E. coli* and *Salmonella typhimurium* in a tropical estuary. Survival has been assessed in relation to the important self-purifying parameters such as biotic factors contained in the estuarine water, toxicity due to the dissolved organic and antibiotic substances in the water and the sunlight. The results revealed that sunlight is the most important inactivating factor on the survival of *E. coli* and *S. typhimurium* in the estuarine water. While the biological factors contained in the estuarine water such as protozoan's and bacteriophages also exerted considerable inactivation of these organisms, the composition of the water with all its dissolved organic and inorganic substances was not damaging to the test organisms. Results also indicated better survival capacity of *E. coli* cells under all test conditions when compared to *S. typhimurium* (Chandram and Hatha, 2005).

The potential use of bacteriophages as indicators of faecal pollution on different types of water has been studied recently (Mandilara *et al.,* 2006). Sunlight inactivation rates of somatic coliphages, F-specific RNA bacteriophages (F-RNA phages), and faecal coliforms were compared in seven summer and three winter survival experiments (Sinton *et al.,* 1999). The consistently superior survival of somatic coliphages suggests that they warrant further consideration as faecal, and possibly viral, indicators in marine waters.

In contrast to the phages of heterotrophic hosts, light can play a key role in all aspects of the life cycle of phages infecting ecologically important marine unicellular cyanobacteria of the genera *Synechococcus* and *Prochlorococcus.* Phage adsorption, replication, modulation of the host cell metabolism, and survival in the environment following lysis, all exhibit light-dependent components. The analysis of cyanophage genomes has revealed the acquisition of key photosynthetic genes during the course of evolution, such as those encoding central components of the light harvesting apparatus (Clokie and Mann, 2006). In another study, quantitative RT-PCR was used to monitor changes in levels of transcripts encoding chaperones and stress-associated proteases in three cyanobacterial strains that inhabit different ecological niches: the freshwater strain *Synechocystis* sp. PCC 6803, the marine high-light-adapted strain Prochlorococcus MED4 and the marine low-light-adapted strain Prochlorococcus MIT9313. Levels of transcripts encoding stress-associated proteins were very sensitive to changes in light intensity in all of these organisms, although there were significant differences in the degree and kinetics of transcript accumulation (Mary *et al.,* 2004).

2.2.2 Temperature

The survival of *E. coli, Salmonella typhi, Shigella sonnei*, poliovirus type 1 and a parvovirus (Minute Virus of Mice) was determined in seawater. Seeded seawater was incubated in the laboratory at 6, 12, 20 and 28°C for up to 40 days. In-situ survival studies were done seasonally (winter, spring, summer and fall) using seeded microbial dialysis equipment placed in the Atlantic Ocean off coastal North Carolina at water depths of 3-10 m. In laboratory studies all test microbes survived longer at lower temperatures with typical times for 90% inactivation (T_{90}) of 1-3 days at the highest temperature and > 10 days at the lowest temperature. Of the microbes tested, *E. coli* survived least well while *S. typhi* and *Sh. sonnei* survived similar to or greater than enteric viruses. Parvovirus survival was similar to that of poliovirus. Under in-situ conditions, *E. coli* also survived least well of all microbes tested with T_{90} values of 0.9-3.9 days depending upon season. All other test microbes had generally similar survival times. Overall, microbial survival in seawater was greater under laboratory conditions than under in-situ conditions. There was no clear association between microbial survival and water temperature (Wait and Sobsey, 2001).

In another study, the behaviour of *Aeromonas hydrophila* in nutrient-poor filter-sterilized seawater was investigated at 23 and 5°C with respect to its growth phase. At both temperatures, the culturable *A. hydrophila* population declined below the detection level (0.1 c.f.u. ml (-1)) after 3-5 weeks, depending on the initial physiological state of the cells (Maalej *et al.,* 2004).

Survival, recoverability and sub lethal injury of two strains of *Listeria monocytogenes*, on exposure to sea water at 12.8 or 20.8ºC was determined using in situ diffusion chambers.

L. monocytogenes challenged at 58°C showed an apparent increase in heat resistance after exposure to sea water at 20.8°C for 7 days (D58 = 2.64 min) compared with before exposure (D58 = 1.24) (Bremer *et al.,* 1998).

To determine the fate of *Legionella pneumophila* in the environment, filtered and unfiltered river water and seawater microcosms, incubated at 4°C and 26°C, were inoculated with [3H] thymidine-labeled *L. pneumophila* cells. Immunofluorescent microscopy, direct fluorescent antibody staining, and acridine orange direct counts were employed besides cultures with the standard method on BCYE. To assess effects of grazing on *Legionella spp,* a duplicate set of samples was filtered through 2.0-microns Nuclepore filters to trap large protozoa. Over the test period, in the microcosms incubated at 4°C, the culturable counts decreased ca. 1 log on BCYE, with no substantial decline in thymidine count. Autoclaved seawater and river water controls held at 15°C also showed no change in thymidine count. At 26°C, a 3-log decline was observed in culturable counts, with ca. 1-log decline in thymidine counts. These results indicate that, although culturability declined by one to three orders of magnitude, when *L. pneumophila* microcosms were incubated at 4°C and 26°C the cells remained metabolically active for extended periods (Paszko-Kolva *et al.,* 1993).

2.2.3 Climate change

Recently a number of studies have linked increases in water temperature due to climate change with changes in microbial populations in surface waters.

In 1996, a major unexplained outbreak of systemic *Vibrio vulnificus* infection erupted among Israeli fish market workers. A possible link between climate change and disease emergence was investigated. Meteorological service data from 1981, the earliest detection and reporting of *V. vulnificus* for the time in Israel, to 1998 for two stations located within the main inland fish farm industry were analyzed. The 1996-1998 summers were identified as the hottest ever recorded in Israel in the previous 40 years. Time series of monthly minimum, maximum, and mean temperatures showed significant increase in the summer temperatures along the 18 years. The highest minimum temperature value was recorded in summer 1996. Lag correlation analysis revealed significant correlations between temperature values and hospital admission dates. The eruption appeared 25-30 days after the extreme heat conditions in summer 1996, at a lag of 3 weeks in summer 1997 while the results for 1998 were at a lag of less than a week. Higher significant results were detected for the daily minimum temperatures in summer 1996 compatible with the disease eruption. These findings suggest that high water temperature might have impacted the ecology of our study area and caused the emergence of the disease, as an effect of global climate change (Paz *et al.,* 2007).

The worldwide increase of eutrophication and global warming has caused wide temperate and oligotrophic sea districts, which are becoming able to host tropical species, even with high food demands. The algal cysts transferred via cargo or in the great oceanic streams, germinate in new environments now favourable for temperature and nutrients, often causing extended blooms. Toxic species has invaded the coasts of several Mediterranean Countries, including Italy in the past few years. Summer blooms of the marine species *Ostreopsis ovata* followed by aerosols toxic for tourists and bathers standing on the shore have been recently noticed in several regions of Italy, pointing out the problems of prevention and risk managing (Salvatore *et al.,* 2006).

Sea water pH ranges between 7.5 and 8.5.For E.coli survival acid pH is favourable and sensitivity increases with the increase in Ph (Carlucci and Pramer,1960).

2.2.4 Competition

Various studies indicate that the main predators of bacteria in the marine environment are protozoa (Davies *et al.,* 1995; Gonzalez *et al.,* 1992; Barcina *et al.,* 1997). In a recent study the objective was to investigate whether aquatic microbiota can limit its survival and recovery of *Vibrio vulnificus* serovar E (VSE) from water samples. A set of preliminary experiments of survival in microcosms containing natural seawater and water from eel farms showed that the persistence of this pathogen was mainly controlled by grazing, and secondarily by bacterial competition. The bacterial competition was further analysed in artificial seawater microcosms co-inoculated with selected virulent serovar E (VSE) strains and potential competitors. Competitors included *V. vulnificus* biotype 1 isolates and strains of selected species that can grow on the selective media designed for *V. vulnificus* isolation from water samples. Evidences of bacterial competition that was detrimental for VSE recovery were recorded. Thus, some species produced a deleterious effect on VSE strains under starvation, and others were able to use the resources more efficiently under nutrient input. These results suggest that an overgrowth of more efficient competitor bacteria in conventional media used for isolation of *V. vulnificus* could mask the recovery of VSE strains (Marco-Noales *et al.,* 2004).

2.2.5 Sedimentation

The fate of indicator organisms (faecal coliform, *E. coli*, and enterococci) from urban stormwater runoff associated with estuarine sediments and stormwater suspended particles was examined (Jeng *et al.,* 2005). The research found a significant increase of faecal coliform, *E. coli*, and enterococci in the estuarine sediments at study sites following a given stormwater event. The sedimentation mechanism was linked to an increase in indicator organisms in the estuarine sediments. These indicator organisms attached to stormwater suspended particles, which then settled on the bottom sediment. A higher percentage of attachment onto stormwater suspended particles was observed for *E. coli* as compared to enterococci and faecal coliform. Among the three organisms, enterococci preferentially attached to the particles with diameters from 10 microm to 30 microm, while faecal coliform and *E. coli* had a broader distribution. Estuarine sediments were found to prolong survival of indicator organisms for at least seven days prior to reduction to background levels.

The distribution of *Clostridium botulinum* serotypes A, B, E and F in aquatic environments of the Baltic Sea and Finnish mainland was examined. A total of 110 samples were tested with a neurotoxin-specific PCR assay. *Cl. botulinum* type E was found in 81% of sea and 61% of freshwater samples. No other toxinotypes were found. Spore counts were quantified by the most probable number method, *Cl. botulinum* type E kg (-1) averaging 940 in sea and 370 in freshwater samples. The overall prevalence and spore counts of *Cl. botulinum* type E in aquatic sediments correlated significantly with offshore bottom oxygen content, depth, and bioturbation activity, whereas there was no correlation with bottom water temperature. These findings indicate the possibility of *Cl. botulinum* type E multiplication or at least, suitable conditions for spore survival, in anoxic sediments (Hielm *et al.*, 1998).

The viability of *Vibrio fluvialis* in seawater microcosms, with and without sediment was investigated. The strain survived as culturable bacteria for at least 1 year and the expression of its virulence factors was maintained. In microcosms containing sediment .*V fluvialis* was more stable. Viable but nonculturable (VBNC) cells of *V. fluvialis* were able to resuscitate to the culturable state up to 6 years of incubation in marine sediment. These cells recuperate their initial biochemical characteristics after 3 months of incubation in marine broth. Amplified 16S ribosomal DNA (rDNA) restriction analysis (ARDRA) was used to confirm that it is the same strain of *V. fluvialis* which resists in all microcosms during a long period of time (Amel *et al.*, in press).

2.2.6 Salinity

The role of salinity in the survival mechanisms of faecal micro-organisms in the marine environment is not well established. Shiga toxin-producing *E. coli* (STEC) O157 was investigated with respect to its halotolerance and whether it can survive in marine water. STEC O157 could multiply in a medium containing 5% NaCl and in sterilized marine water, and could survive in unsterilized marine water for at least 15 days. The shiga toxins stx, comprising stx1 and stx2 were detected from marine water samples by PCR (Miyagi *et al.,* 2001).

The effects of various concentrations of sodium chloride solutions (0.1%-3%) and different temperatures (4, 10, 20, 30 and 37°C) on survival of *Legionella pneumophila* were investigated. It was found that at temperatures between 4 and 20°C, *L. pneumophila* organisms survived in salt solutions up to 3% NaCl. Only the combination of high temperatures, i.e. 30 and 37°C, with NaCl concentrations over 1.5%, reduced cell numbers significantly. It was interesting to note that the addition of small amounts of NaCl (0.1%-0.5%) enhanced survival of *L. pneumophila*, suggesting a protective effect of NaCl (Heller *et al.,* 1998).

3. HEALTH IMPLICATIONS OF RECREATIONAL WATER USE

As discussed in section 1 recreational waters in any location generally contain a mixture of pathogenic and non-pathogenic micro-organisms originating from sewage contaminated by human excreta and diffuse sources from surface contamination by animal excreta. Available evidence suggests that the most frequent adverse health outcome associated with exposure to faecally-contaminated recreational water is enteric illness, such as self-limiting gastroenteritis, which may not be formally recorded in the disease surveillance system, and may be of short duration. A number of other conditions are possible such as respiratory illness or skin infections.

The principal burden of disease is via the faecal-oral route. The number of micro-organisms that may cause disease or infection depends upon the specific pathogen, the form in which it is encountered, the conditions of exposure and the host's susceptibility and immune status. For viral and parasitic protozoan illnesses, this dose may be very few viable organisms (Fewtrell *et al.,* 1994; Haas *et al.,* 1999).

The types and numbers of pathogens in sewage will vary depending on the incidence of disease in the contributing human and animal populations and the seasonality of infections. Numbers will therefore vary across the Mediterranean region and will be different from northern Europe and other parts of the world. The Mediterranean is heavily used by tourists who on the one hand may influence the pathogen numbers in the sewage and on the other hand be susceptible to local pollution.

Despite most illnesses contracted through recreational waters being mild, diseases with a range of severities may also occur and diseases that are normally mild and self-limiting can become severe in susceptible populations, such as immunocompromised persons, the elderly and the very young.

A number of outbreaks have been attributed to recreational waters (Table 2) The most frequent adverse health outcome associated with exposure to faecally contaminated recreational water is enteric illness. A cause-effect relationship between faecal or bather-derived pollution and acute febrile respiratory illness (AFRI), which is a more severe health outcome than gastroenteritis, has also been shown (WHO, 2003).

The initial classification of a recreational water environment is based upon the combination of evidence for the degree of influence of (human) faecal material alongside counts of suitable faecal index bacteria (a microbial quality assessment). Information to be collected during sanitary inspections should cover at least the three most important sources of human faecal contamination of recreational water environments for public health purposes: sewage, riverine and bather contamination (refer to section 1). Where human inputs are minimal, investigation of animal faecal inputs should be explored. In addition to micro-organisms introduced to recreational waters through human or animal faecal contamination, a number of pathogenic micro-organisms are indigenous to such areas or, once introduced, are capable of colonizing the environment (WHO, 2003).

Table 2
Outbreaks associated with recreational waters in the USA, 1985-1998[a]

Etiological agent	Number of cases	Number of outbreaks
Shigella spp.	1780	20
Escherichia coli O157:H7	234	9
Leptospira sp.	389	3
Giardia lamblia	65	4
Cryptosporidium parvum	429	3
Norwalk-like viruses	89	3
Adenovirus 3	595	1
Acute gastrointestinal infections (no agent identified)	1984	21

[a] WHO, 2003

Water contact time is a prime factor influencing the amount of exposure to pathogens in water. The longer a person is in the water the more they can be exposed to pathogens in the water through ingestion, inhalation or penetration of the skin (e.g., schistosomiasis). The US EPA estimates that 100 ml of water enters the mouth and nasopharynx during a typical swimming episode (US EPA, 1999). Review of the literature did not reveal any published estimates of the quantities of water ingested during recreational water activities other than swimming or provide estimates of average immersion times.

Some activities are likely to pose greater risk of water ingestion than others. The British Sub Aqua Club for example estimates that in winter the average length of a scuba dive is between 20 minutes and 30 minutes but in summer it can be more than one hour (Alistair Reynolds, British Sub Aqua Club Technical Manager, personal communication, 2001). The average volume of water consumed during a typical dive is not known. A study of scuba divers from New York City's police and fire departments indicated an association between scuba diving and gastrointestinal illness (Anonymous, 1983). The divers reported ingesting small quantities of water while swimming at the surface and while using mouthpieces that had dangled in the water before use. Stool samples revealed 12 cases of gastrointestinal parasites - five of *Entamoeba histolytica* and seven of *Giardia lamblia*. One bacterial culture was positive for *Campylobacter*. Twenty-three non-diving fire-fighters had stools examined for parasites; none had *G. lamblia* or *E. histolytica*.

In recent years the popularity of activities which involve contact with water has grown and the increasing availability of the wet suit has altered the public use of recreational water especially in temperate regions with colder water. Prolonged periods of immersion are now becoming normal and activity occurs throughout the year and not just during the bathing seasons. Many gastrointestinal infections occur on a seasonal basis and therefore users will be exposed to different types of pathogens in the water. The density of users (bather-loads) at smaller recreational water bodies, especially where there is limited water turnover, may be a significant factor in the user-to-user transmission of disease. The personal hygiene of recreational water users while in the water (which may also significantly alter the quality of the water) is also a concern. A number of *Cryptosporidium* outbreaks in pools are thought to have been caused by swimmers who have had 'faecal accidents' (Lee and Joseph, 2002). In addition, certain activities that increase the likelihood of ingestion of water, e.g. surfing, may lead to higher levels of risk (WHO, 2003).

Skin abrasions or cuts may contribute to recreational water-associated infections. Many environmental bacteria such as species of *Pseudomonas*, *Aeromonas*, and halophilic vibrios are opportunistic pathogens that may cause wound infections. In some cases, these infections can be life-threatening, e.g. *Vibrio vulnificus* (Chang *et al.* 1997).

The number of micro-organisms (dose) that may cause infection or disease depends upon the specific pathogen, the form in which it is encountered, the conditions of exposure and the host's susceptibility and immune status. For viral and parasitic protozoan illness, this dose might be very few viable infectious units (Fewtrell *et al.,* 1994; Teunis *et al.,* 1996; Haas *et al.,* 1999; Okhuysen *et al.,* 1999; Teunis *et al.,* 1999). In reality, the body rarely experiences a single isolated encounter with a pathogen, and the effects of multiple and simultaneous pathogenic exposures are poorly understood (Esrey *et al.,* 1985).

Pathogenic micro-organisms encountered in the marine environment include bacteria, protozoa and viruses. The most important species with emphasis to their association with potential health risks due to swimming in the marine environment are briefly described below.

3.1 Bacteria

3.1.1 Salmonella spp

The primary habitat of the *Salmonella spp* is the intestinal tract of animals and humans. For epidemiological purposes Salmonellae are classified in 3 categories; those that infect the humans only (*S. typhi*, *S. paratyphi A and S. paratyphi C*); the host adapted serotypes (i.e. *S. gallinarum*); and unadapted serotypes that include most waterborne and foodborne serotypes.

Distribution is worldwide. Over the past three decades, practically all countries in Europe have reported a sharp rise in salmonellosis incidence (including foodborne outbreaks). The same pattern was observed in a number of countries in the Eastern Mediterranean Region and south-east Asia Region (WHO, 1997). Unfortunately there was a ten-fold increase in the number of human cases of multi-drug resistant *S. typhimurium* DT 104 in the six-year-period between 1990 and 1996 rising from 300 to 3500 cases per year in England and Wales. This specific strain was second only to *S. enteriditis* as the most common salmonella in humans in England and Wales. A number of studies from throughout the world have investigated the incidence and survival of Salmonella in rivers, lakes, coastal water and beach sediments (Medema *et al.,* 1995; Johnson *et al.,* 1997; Polo *et al.,* 1998). In these environments some, but not all, strains of Salmonella are pathogenic. Nevertheless *S. typhi* does not survive well in polluted or warm waters but survival is extended in sediments (Holden, 1970).

The presence of Salmonella and its relationship with indicator organisms of faecal pollution, such as total coliforms, faecal coliforms and faecal streptococci, was studied at two marine zones in Portugal. Seventeen different Salmonella serotypes were isolated and identified; *S. virchow* was the most frequently isolated (21.6%). In addition, a high percentage (35.1%) was recorded for some Salmonella serotypes of clinical significance, namely *S. enteriditis, S. infantis, S. typhimurium* and *S. virchow*. In any of the samples from the two zones Salmonella was not detected in the absence of any of the indicator organisms. However, the incidence of Salmonella as a function of indicator concentration intervals established by the EC bathing water standards (EC, 1976) was 0, 10 and 19.3% at guide values of total coliforms, faecal coliforms and faecal streptococci, respectively in the Faro samples (south of Portugal). In contrast, Salmonella incidence rates of 37.5, 36.4 and 33.3% were recorded at the corresponding guide values the Caminha samples (north of Portugal). No significant correlations ($p>0.005$) were obtained between Salmonella and the indicators at the sampling stations; however, total coliforms and faecal streptococci were the indicators most closely related to Salmonella in Caminha and Faro samples, respectively. Survival experiments in *Escherichia coli*, *Enterococcus faecalis* and *S. typhimurium*, using diffusion chambers, were performed to verify whether the lack of correlation between indicators and Salmonella was due to different inactivation rates in seawater. The results indicate that survival percentages of the three micro-organisms tested were similar after 48 h of exposure to seawater (Catalao Dionisio *et al.,* 2000).

3.1.2 E.coli O157:H7

In recent years, *E. coli* O157:H7 has been increasingly linked to foodborne and waterborne gastroenteritis. The most serious consequence associated with *E. coli O157:H7* is Haemolytic Urinary Syndrome (2-8% of the cases). Children are most commonly affected and many cases have been described (Lansbury and Ludlam, 1997; Rowe *et al.,* 1991). , *E. coli* O157:H7 is a pathogen which is of concern worldwide (Jones, 1999). EHEC was first identified as a human pathogen after two outbreaks occurred in the United States (Konowalchuk *et al.* 1977). Since then outbreaks have been reported from various parts of the world including north America, western Europe, Australia and Asia (Clarke, 2001; Cowden *et al.*, 2001; Elliot *et al.,* 2001; Yamamoto *et al.,* 2001).

Several outbreaks of *E. coli* O157:H7 have been associated with swimming in freshwater. Between 1992 and 1999 1333 cases were reported in Wisconsin, of which 8.1% were related to recreational waters. (Proctor and Davis, 2000). The Netherlands, Scotland, UK and the States of Oregon and Atlanta, Georgia are among the countries that have reported clusters of cases (Pond, 2005).

3.1.3 Leptospira spp

Leptospira spp is an important cause of waterborne infections in the tropical, developing countries, even though the disease is not unknown in the temperate zones. The main source of leptospires is the renal tubes of a carrier animal. Mild forms of leptospirosis (also known as Weil's disease or haemorrhagic jaundice) range from a febrile incapacitating illness lasting between 10 and 20 days, consisting of severe muscle pains, meningism and mild renal incapacity, to a barely detectable subclinical infection. Severe forms are frequently fatal if untreated; (Arean, 1962; De Brito *et al.,* 1979).

There is clear epidemiological evidence and outbreak data linking cases of leptospirosis to persons using water for recreational purposes. However, *Leptospira* spp. is usually associated with animals that urinate into surface waters, and swimming-associated outbreaks attributed to *Leptospira* are rare. The acute illness is considered moderately severe but is often prolonged. There is a moderate probability of developing long-term sequelae.

3.1.4 Shigella spp

Shigella spp is the cause of bacillary dysentery. Shigellosis is primarily a paediatric disease. Watery or bloody diarrhoea, abdominal pain, fever, and malaise are caused by *S. dysenteriae* type 1. Abdominal cramps, fever and watery diarrhoea occur early in the disease. Dysentery occurs during the ulceration process, with high concentrations of neutrofils in the stools.

Infection with *Shigella* spp is not often spread by waterborne transmission, even though major outbreaks resulting from such transmission have been described. A limited number of cases of shigellosis associated with recreational waters were found in the literature, mostly related to exposure to fresh waters.

3.1.5 Campylobacter spp

Campylobacter spp has been isolated form a number of environments. *C. jejeuni and C. coli* produce a cholera- like enterotoxin and they are commonly associated with infective diarrhoea in most developing countries. Common symptoms are cramps, abdominal pain, and diarrhoea with or without blood, chills and fever. The common route of transmission is infected meat and water.

C. jejuni has been increasingly found in sewage and isolated form surface waters (Lambert *et al.,* 1998, Eyles *et al.*, 2003), including EU bathing waters (Brennhovd *et al.,* 1992, Arvanitidou *et al.,* 1995). Campylobacters are transmitted to the bathing waters through untreated sewage and animals shedding this pathogen through their faeces. Thermophilic campylobacters have been isolated from Jones (1990) and in intertidal sediments on 3 bathing beaches in U.K (Obiri-Danso and Jones, 2000). Counts were higher in winter months. Beach sediment may therefore act as a reservoir for campylobacters in winter months.

3.1.6 Vibrio cholerae

In developing countries cholera is one of major diseases associated with the consumption of sewage-contaminated shellfish. *Vibrio cholerae* was discovered by Koch in Egypt during the 1883 epidemic. Cholera is an acute intestinal infection caused by the bacterium *V. cholerae.* It produces an enterotoxin that causes copious, painless, watery diarrhoea that can quickly lead to severe dehydration and death if treatment is not promptly given. Vomiting also occurs in most patients. Most persons infected with *V. cholerae* do not become ill. When illness does occur, more than 90% of episodes are of mild or moderate severity and 10% of ill persons develop typical cholera with signs of moderate or severe dehydration.

The vibrio responsible for the seventh pandemic, now in progress, is known as *V. cholerae* O1, biotype El Tor. The current seventh pandemic began in 1961 when the vibrio first appeared as a cause of epidemic cholera in Celebes (Sulawesi), Indonesia. The disease then spread rapidly to other countries of eastern Asia , it invaded West Africa, which had not experienced the disease for more than 100 years, and in 1991 cholera struck Latin America, where it had also been absent for more than a century. Within the year it spread to 11 countries, and subsequently throughout the continent.

V. cholerae serogroup O1 has been recognised as the only group causing epidemic cholera until 1992. Some other serogroups could cause sporadic cases of diarrhoea, but not epidemic cholera. Late in 1992, however, large outbreaks of cholera began in India and

Bangladesh caused by a previously unrecognized serogroup of *V. cholerae*, designated O139, synonym Bengal. Isolation of this vibrio has now been reported from 11 countries in South-East Asia. It is still unclear whether *V. cholerae* O139 will extend to other regions and careful epidemiological monitoring of the situation is being maintained (www.who.int).

Cholera is mainly a foodborne and waterborne disease *V. cholerae* is often found in the aquatic environment and is part of the normal flora of brackish water and estuaries. It is often associated with algal blooms (plankton), which are influenced by the temperature of the water. Human beings are also one of the reservoirs of the pathogenic form of *V. cholerae.* Brisou *et al.* (1962) isolated 44 strains of Vibrios from the Algerian coast.

3.1.7 Staphyloccocus aureus

Staphyloccocus aureus is a pathogen associated with skin, ear and mucus membranes infections and intoxications and is commonly related to food poisoning (UNEP/MAP/WHO, 1996).

The origin of the pathogen in seawater has been attributed to human activity as strains are found to be shed by bathers under all conditions of swimming (Robinson and Mood, 1996; WHO, 2003). In a Greek survey, all indicators had a positive association with *Staphyloccocus aureus* counts in sewage contaminated bathing waters (Efstratiou *et al.*, 1997). In a number of studies a significant correlation appears between the number of swimmers present on the beach and *Staphyloccocus aureus* counts in bathing bather samples. (Papadakis *et al.*, 1997). According to other studies, *Staphyloccocus aureus* predominate over other flora in the beach sand (Dowidart and Abdel-Monem, 1990). Investigations carried out along the Tyrrhenian coast, Italy, shown higher densities of *Staphylococcus spp* in sand of areas with breakwaters than in sands found in open seas (Bonadonna *et al.*, 1993).

3.1.8 Aeromonas spp

Aeromonas spp. are natural inhabitants of aquatic environments and are ubiquitous in surface fresh and marine waters, with high numbers occurring during the warmer months of the year. Clinical isolation of these microbes presents the same seasonal distribution. Numbers may be high in both polluted and unpolluted habitats with densities ranging from <1 to 1,000 cells per ml. Sewage can also contain elevated numbers (10^6-10^8 cells per ml) of aeromonads. *Aeromonas* has been found to have a role in a number of human illnesses including gastroenteritis. Cases of wound infections in healthy people associated with recreational water have been described, as have cases of pneumonia following aspiration of contaminated recreational water.

3.1.9 Vibrio vulnificus

V. vulnificus is a natural inhabitant to the marine environment worldwide (Montanari *et al.*, 1999). It is capable of causing necrotising wound infections, gastroenteritis, and primary septicaemia. The symptoms and severity of disease depend on the type of infection.

Health problems associated with exposure to *V. vulnificus* might be underreported. The majority of human infections reported in the literature are through consumption of contaminated raw or undercooked seafood. However, infection is also through the contamination of wounds by seawater or marine animals (Hlady and Klontz 1996). Recreational water users with open wounds should therefore be aware of the possibility of infection particularly in summer months in temperate areas when the water temperatures are higher. Between 1997 and 1998, CDC received reports of over 389 cases of culture confirmed *Vibrio* illnesses from the Gulf Coast states, where the majority of cases in the United States have been reported. Among those about whom this information was available, 37% were hospitalised and 7% died. *V. vulnificus* accounted for 89% of the reported deaths. Of the total illnesses reported 16% were classified as wound infections, in which the patient incurred a wound before or during exposure to seawater or seafood drippings, and *Vibrio* spp. was subsequently cultured from the blood, wound or normally sterile site.

V. vulnificus quite often occurs in marine and estuarine environments. Evidence exists for the association of recreational use of water and infection with *V. vulnificus* where the user has a pre-existing open wound. Surveillance of *V. vulnificus* infections is poor and the number of cases reported is likely to be underestimated.

3.2 Protozoa

3.2.1 Giardia duodenalis (previously known as G. lamblia)

Giardia has become recognised as one of the most common causes of waterborne disease (drinking and recreational) in humans. Infection with *G. duodenalis* begins with asymptomatic cyst passage, acute usually self-limited diarrhoea and a chronic syndrome of diarrhoea, malabsorption, and weight loss.

Giardia has been transmitted during swimming (Johnson *et al.,* 1995) and *Giardia* cysts have been isolated readily from surface water samples (Le Chevallier *et al.,* 1991), from water samples taken from coastal beaches (Ho and Tam, 1998; Lipp *et al.,* 2001a), rivers used for recreational activities (Bing - Mu *et al.,* 1999) and swimming pools (Fournier *et al.,* 2002).

3.2.2 Cryptosporidium parvum

Cryptosporidiosis is generally more serious in the immunocompromised. Infection with *C. parvum* results in severe watery diarrhoea which lasts between several days and two to three weeks in previously healthy persons.

Cryptosporidiosis has been associated with swimming in fresh recreational water exposure but to our knowledge no cases related with exposure to the marine environment have been reported.

3.3 Viruses

3.3.1 Hepatitis A

Hepatitis A (HAV) infection first symptoms consist of fever, nausea, loss of appetite, abdominal pain and mild gastrointestinal upset, followed by jaundice. In young children the disease is often asymptomatic and the severity of disease increases with age. The first signs of jaundice are a darkening of the urine and a lightening of stools. The patient may also show signs of a yellowing of the eyes and an enlarging of the liver. The patient probably remains infectious for seven days after the start of the jaundice (Hunter, 1998).

A number of studies have isolated HAV from surface waters which could be used for recreational purposes and therefore may pose a potential health risk. Several studies have reported HAV in the effluent of treatment plants implying a potential risk posed by the discharge of viruses (Hugues *et al.,* 1988). To date no cases have been associated to swimming in the marine environment but unreported cases might have occurred.

3.3.2 Hepatitis E virus

Hepatitis A (HEV) is an additional to HAV cause of waterborne hepatitis with onset of fever, malaise, nausea and abdominal discomfort, followed by the development of jaundice a few days later. Infection in very young children is usually mild or asymptomatic; older children are at risk of symptomatic disease. The disease is more severe in young to middle aged adults.

HEV is mainly transmitted via the enteric route and therefore contamination of sewage with animal-derived faeces may represent another important source of transmission of such viruses to humans and animals consuming contaminated water. This may have implications for recreational water management. Most cases of acute HEV in the United States, central and western Europe have been reported amongst travellers returning from high HEV-endemic areas, although this is not always the case (Zuckerman, 2003). It is suspected that in some countries the cases of HEV infection could be causatively related to the consumption of shellfish cultivated in sewage-polluted waters (Balayan, 1993). The possibility of transmission through swimming in polluted marine environment cannot be excluded.

3.3.3 Human adenovirus

Adenoviruses are common causes of fevers, upper respiratory tract symptoms and conjunctivitis and produce infections that are usually mild and self limiting.

In seawater the enteric adenoviruses have been shown to be more stable than either polio 1 or HAV and sometimes are detected at higher levels in polluted waters (Crabtree *et al.,* 1997). This suggests that the enteric adenoviruses may survive for prolonged periods in water, representing a potential route of transmission (Enriquez and Gerba, 1995).

3.4 Severe outcomes from bathing in recreational waters

There are a number of waterborne pathogens that can cause illnesses with severe outcomes, as discussed in section 3.0. Severe outcomes occurring among recreational water users using sewage-polluted waters are generally in those that are short-term visitors from regions with low endemic disease incidence (WHO, 2003). Very few epidemiological studies have tried to quantify the risks of acquiring more serious illnesses although it is biologically plausible that viruses, bacteria and protozoa associated with more severe health outcomes may be transmitted through the use of contaminated water.

Secondary outcomes resulting from a previous infection by the pathogen may also be evident. The primary disease symptoms and sequelae associated with these organisms are given in Table 3.

The evidence that micro-organisms or their products are directly or indirectly associated with sequelae range from convincing to circumstantial, mainly because it is unlikely that most of the epidemiological studies conducted to establish a link between bathing and illness do not address the more severe health outcomes or possible sequelae. This is likely to be due to the low occurrence of severe health outcomes in the temperate regions where the majority of studies have been conducted, and because investigations of rarer outcomes usually require larger study groups. In addition, host symptoms caused by a specific pathogen or product are often wide-ranging and difficult to link with a specific incident, especially as the time of onset of the sequelae may vary (Pond, 2005). Epidemiological investigations of *Cryptosporidium parvum* and *Giardia lamblia* by for example Keene *et al.* (1994), Kramer *et al.* (1998) and Barwick *et al.* (2000) suggest that the source of the outbreak was usually the bathers themselves.

Table 3
Primary disease symptoms and sequelae associated with some micro-organisms that may be found in recreational waters

Pathogen	Primary disease symptom	Sequelae
Campylobacter spp.	Diarrhoea, cramping, abdominal pain, malaise.	Gullian-Barre syndrome, acute motor neuropathy, opthalmoplegia, Reiter's syndrome, infection of various organs and blood.
Salmonella typhi	Typhoid, malaise fever, aches, abdominal pain, diarrhoea, constipation, delirium.	Septic arthritis, Reiter's syndrome, pyogenic lesions, intracranial abscess, osteomyelitis.
Shigella dysenteriae	Severe abdominal pain, watery diarrhoea or stools containing blood	Aseptic or reactive arthritis.
E. coli O157	Severe bloody diarrhoea and abdominal cramps	Haemolytic uraemic syndrome Thrombotic thrombocytopenic purpura
Cryptosporidium spp.	Diarrhoea, mild abdominal pain, mild fever	Loss of fluids, anorexia, malabsorption of nutrients.
Viral hepatitis	Malaise, lassitude, myalgia, fever and sometimes jaundice	Idiopathic autoimmune chronic active hepatitis

Source: adapted from Pond (2005)

3.5 Correlation between adverse health outcomes and recreational water use

As discussed, both mild and severe infections and illnesses associated with recreational water use are difficult to detect through routine surveillance systems and to attribute to water use. However, there is a significant body of epidemiological evidence derived from studies from around the world and dating from the 1950's, which concludes that there is an association between gastrointestinal symptoms, AFRI and indicator-bacteria concentrations (Table 4). This can result in a significant burden of disease and economic loss to the affected region.

Dose-response relationships derived from the studies of Cabelli *et al.* (1982) and later by Kay *et al.,* (2001) and others link self-reported and normally self-limiting, gastroenteritis with water quality as indexed by the faecal coliform indicators identified in the EU bathing water Directive (EC, 1976).

In order to assist the process of development of the WHO Guidelines for safe recreational water environments (WHO, 2003), Pruss (1998) reviewed 22 epidemiological studies and reported the dose-response curves from the results (Figure 1). The studies reviewed by Pruss (1998) were selected on the grounds that they met the following criteria: health outcomes are clearly related to water quality; associations of interest are sufficiently documented and can be calculated from the reported data; exposure or outcome assessment does not differ significantly among exposure or outcome groups; study population is sufficiently large (more than three diseased per exposure groups); response rate is more than 50%; water of exposure is not artificially chlorinated.

Table 4
Major epidemiological studies investigating the health effects from exposure to recreational water conducted between 1953 and 2006 (Pruss, 1998)

First author	Year	Country	Type of water
Wiedenmann	2006	Germany	Fresh
Fleisher*	1996	United Kingdom	Marine
Haile*	1996	United States	Marine
Van Dijk	1996	United Kingdom	Marine
Van Asperen	1995	The Netherlands	Fresh
Bandaranayake*	1995	New Zealand	Marine
Kueh*	1995	Hong Kong	Marine
Medical Research Council*	1995	South Africa	Marine
Kay*	1994	United Kingdom	Marine
Pike*	1994	United Kingdom	Marine
Fewtrell	1994	United Kingdom	Fresh
Von Schirnding	1993	South Africa	Marine
McBride	1993	New Zealand	Marine
Corbett	1993	Australia	Marine
Harrington	1993	Australia	Marine
Von Schirnding	1992	South Africa	Marine
Fewtrell*	1992	United Kingdom	Fresh
Alexander	1991	United Kingdom	Marine
Jones	1991	United Kingdom	Marine
Balarajan	1991	United Kingdom	Marine
UNEP/WHO*	1991	Israel	Marine
UNEP/WHO*	1991	Spain	Marine
Cheung*	1990	Hong Kong	Marine
Ferley*	1989	France	Fresh
Lightfoot	1989	Canada	Fresh
New Jersey Department of Health	1989	United States	Marine
Brown	1987	United Kingdom	Marine
Fattal, UNEP/WHO*	1987	Israel	Marine
Philipp	1985	United Kingdom	Fresh
Seyfried*	1985	Canada	Fresh
Dufour*	1984	United States	Fresh
Foulon	1983	France	Marine
Cabelli*	1983	Egypt	Marine
El Sharkawi	1982	Egypt	Marine
Calderon	1982	United States	Marine
Cabelli*	1982	United States	Fresh and Marine
Mujeriego*	1982	Spain	Marine
Public Health Laboratory Service	1959	United Kingdom	Marine
Stevenson*	1953	United States	Fresh and Marine

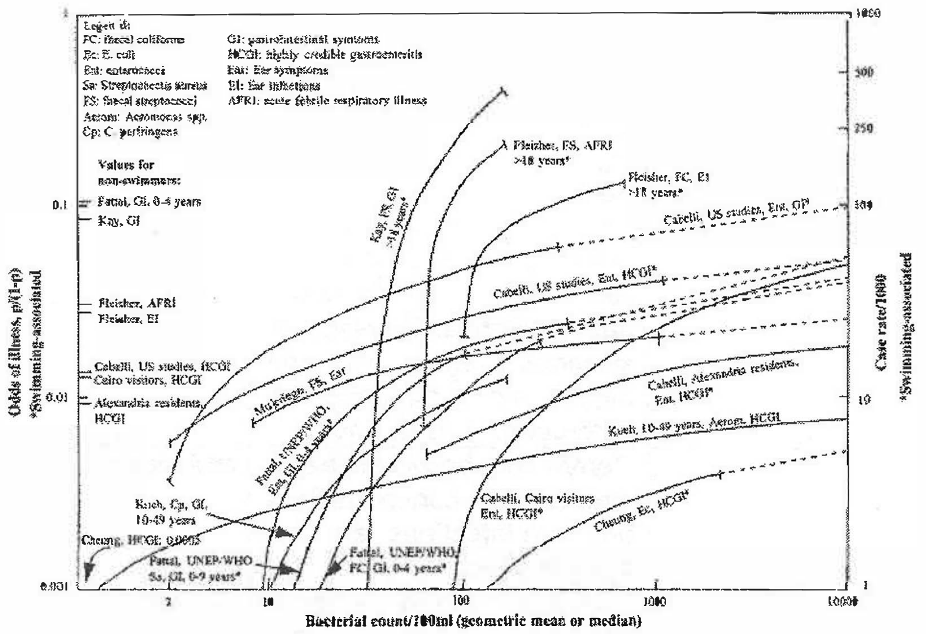

Figure 1. Dose-response curves reported by selected epidemiological investigations in recreational waters, reviewed as part of the development of the WHO Guidelines for safe recreational water environments (Pruss, 1998)

Of the twenty-two papers selected, 18 were based on prospective cohort studies, two were based on retrospective cohort studies and two were based on a randomised-controlled trial. Water quality in the selected studies was assessed by measuring indicator micro-organisms, usually faecal bacteria. The most commonly used indicators are enterococci, *Escherichia coli*, and faecal coliforms. A few studies measured pathogenic micro-organisms. Water quality was measured on a daily (some at time of exposure) basis for 11 of the selected studies and analysed accordingly. The remainder mainly used seasonal water quality means of the selected beaches for association with outcomes. Potential confounding factors considered in the studies included food and drink intake, age, sex, history of certain disease, drug use, personal contact, additional bathing, sun, socio-economic factors, etc. Different studies controlled for different numbers of confounding factors.

The review undertaken by Pruss (1998) included several studies from the Mediterranean – Mujeriego et al. (1982), El Sharkawi and Hassan (1982), Cabelli *et al.* (1983), Foulon (1983), Fattal *et al.* (1987) and Ferley *et al.* (1989). These and other epidemiological studies have been reviewed by the previous technical report of the Mediterranean Action Plan (UNEP/MAP/WHO, 1996). Their findings are summarised in Table 5.

The conclusion from Pruss (1998) was that many studies world-wide (including the Mediterranean) show a dose-response relationship linking faecal indicator concentrations in bathing waters to reports of minor illnesses (gastroenteritis, acute febrile respiratory illness and ear infections) in the exposed population. Relative risk (RR) values for swimming in polluted water versus staying on a beach without entering the water were often significant and usually ranged between 1 and 3.

Table 5
Summary findings from epidemiological studies carried out in the Mediterranean

Country of study	Main findings	Reference
Egypt	Strong association between highly-credible gastrointestinal symptoms and concentrations of enterococci and *E. coli.*	Shuval (1986)
Israel	Association between gastrointestinal illnesses in swimmers aged 0-4 and higher concentrations of enterococci and *E. coli.*	Fattal and Shuval (1989)
Spain	Correlation between faecal streptococci concentrations and skin infections, ear and eye infections.	Mujeriego *et al.*, 1983
Spain	Enteric symptoms higher in swimmers than non-swimmers on more polluted beaches; Dermatological and skin symptoms higher in swimmers compared to non-swimmers irrespective of water quality.	Borrego *et al.*, 1991
France	Higher incidence of conjunctivitis, skin infections and abdominal discomfort in bathers but no correlation with water quality.	Foulon (1983)
Turkey	Morbidity rates for gastrointestinal symptoms reported by swimmers ranged from 7.2% in less polluted beaches to 9.5% on more polluted beaches.	Kocasoy (1989)
Italy	Morbidity for enteric symptoms were higher in users of beaches classified as polluted compared to users of non polluted beaches.	Torregrossa *et al.*, 1994

Increased rates of eye symptoms have been reported in swimmers, but this has been shown to be independent of water quality (Pruss, 1998). Similarly, rates of skin infections have been shown to be higher amongst swimmers, but there is no clear association between faecal pollution and skin symptoms (WHO, 2003).

The epidemiological studies conducted to date have shown that existing microbiological standards for bathing waters may not prevent some minor illnesses being transmitted. However, the scientific evidence base is still insufficient to underpin credible health-evidence-based microbial standards for bathing waters. Fleisher *et al.,* (1993) argues that all previously published epidemiological studies of the health effects of bathing in marine waters contaminated with domestic sewage contain three major methodological weaknesses in study design: a failure to control for the substantial amount of temporal and spatial variation in indicator organism densities shown to occur within just a few hours at marine water bathing locations; a failure to relate indicator organism density directly to the individual bather; and a failure to rigorously control for non-water-related risk factors on previously reported associations between bathing in marine waters and illness among such bathers.

Although studies have shown that the general relationship between faecal streptococci and ill health in marine waters remains qualitatively similar in warmer countries, the exact relationship between markers of faecal pollution and risk of illness is unknown. There is considerable evidence that light intensity and temperature have major impacts on the survival of *E. coli* and many enteric pathogens in surface waters and therefore the relationship between indicators and disease risk in northern European studies will not be the same in Mediterranean countries, though it is uncertain whether risk will be greater or less for a given indicator level. It is possible that there might be a range of different risks depending on the immunity of the exposed population and the nature of the pathogens which are associated with faecal indicators in different bathing water environments (e.g. fresh and marine; those receiving human treated effluents and/or animal derived faecal indicators from birds and livestock etc.).

On-going research is establishing the nature and level of the risk associated to swimming in microbiological contaminated waters in the Mediterranean and how to identify how exposure affects risk through the relationship between water quality and health outcome as well as the level of protection afforded by the threshold values for microbial contamination in the new EC Directive (EU, 2006) against values in the original directive (76/160/EEC). http://www.aber.ac.uk/iges/research/epibathe/Summary%20Of%20Epibathe.pdf

4 CURRENT STANDARDS FOR BATHING WATERS WORLDWIDE

4.1 Guidelines and standards

Water quality standards relating to recreational water vary throughout the world. Microbiological standards are intended to protect the health of those bathers that are not already immune to the pathogens from which they are to be protected. They should provide a degree of protection not only to the endemic population but also to tourists which may be completely different in terms of susceptibility to the pathogenic organisms present at the given site (Wiedenmann *et al.,* 2006).

Mediterranean countries in the EC are governed by the EU Directive on bathing water quality (EC, 1976) which has recently been revised (EC, 2006) to take account of the results of epidemiological studies conducted to assess the link between swimming in sewage polluted waters and minor illnesses, as described in section 3.

4.1.1 Mediterranean National legislation

Throughout the world, there is a diversity of quality standards adopted that vary to quite an extent. A number of them are expressed as a permissible mean concentration and a maximum value that should not be exceeded in a given percentage of the samples. For practical purposes, countries can be split in the following major groups: European Union countries, Mediterranean non EU countries, United States of America (USA), Latin American countries, other countries.

The European countries belonging to the European Union are bound by the relevant EU Directives, while the Mediterranean non-EU countries by their common Guidelines. Although the national legislation in some Mediterranean EU member states is based on the relevant Directive, the measures in force are stricter for France, Greece, Italy and Spain (Table 6).

Table 6
Microbiological quality criteria and standards for bathing waters in EU Mediterranean countries

	Total coliforms Per 100 ml	**Faecal coliforms per 100 ml**	**Faecal Streptococci per 100 ml**	***Salmonella* per l Litre**	**Entero-viruses per 10 Litres**	**Remarks**
Cyprus	500 (80%) 10000(95%)	100 (80%) 500 (95%)	100 (90%)	0	0	
France	500 (80%) 10000(95%)	100 (80%) 2000 (95%) ≤ 100 (80%) ≤ 2000 (95%) ≤ 2000 (95%) ≥2000 (5%<n<33%) ≥ 2000 (>33,3%)	100 (90%) ≤ 100 (90%)	0 0 0 0 0	0 0 0 0 0	A Good quality B Medium quality C Poor quality D Bad quality
Greece	500 (80%) 10000(95%)	100 (80%) 500 (95%)	100 (90%)	0	0	
Italy	500 (80%) 2000(95%)	100 (80%) 2000(95%)	100 (90%)	0	0	
Malta		100 (95%) 100 (50%) 1000 (90%)				First class Second class
Slovenia	500 (80%)	100 (80%)	100 (80%)	0	0	Only for special purposes (not legally binding), e.g. blue flag programme
	10000(95%)	2000 (95%)		0	0	
Spain	500 (80%) 10000(95%)	100 (80%) 2000(95%)	100 (90%)	0	0	
EEC Europe	80%<500 95%<10,000	80%<100 95%<2,000	100 (90%)	0	0	Also, Enterococci 90%<100

The Mediterranean non EU countries have agreed on common guidelines, as umbrella standards, and are permitted to use stricter ones (Kamizoulis *et al.*, 2005). It is to be noted that some Mediterranean non-EU countries base their national legislation on the 1976 EU Directive (revised), being stricter than the UNEP/WHO common Guidelines (Table 7).

Table 7
Microbiological quality criteria and standards for bathing waters in Mediterranean non EU countries

	Total coliforms per 100 ml	**Faecal coliforms per 100 ml**	***Escherichia coli* per 100 ml**	**Faecal Streptococci per 100 ml**	**Remarks**
Albania			200 - 400		should not generally exceed value
Algeria					EU legislation
Bosnia-Herzegovina					Standards based on *E.coli*, Faecal enterococci.
Croatia	500(100%)	100 (80%)		100 (80%)	Seawater suitable for bathing
	1000	200		200	Moderately polluted seawater
	>1000				Polluted seawater
					Also EU Directive values are used for special purposes, e.g. blue flag programme
Egypt	500cfus / 100 ml		100 cfus/100ml	100 cfus/100ml	Every two months for all parameters ISO 9308-1 (t.coliform) ISO 7899/2 (f. streptocc.) ISO 9308/1 (*E. coli*)
Israel		200 400 (80%)			geometric mean individual samples
Lebanon	10000 (M) 500 (G)	2000 (M) 100 (G)		100 (G)	Zero tolerance for Salmonella (G) and Enteroviruses (G)
Libya		100 (50%) 1000 (90%)			
Monaco					France – EU legislation
Montenegro	500 (80%) 10000(95%)	100 (80%) 500 (95%)	100 (90%)	0	-
Morocco			100 (80%) 200 (95%)	100 (90%)	
			≤100 (80%) ≤2000 (95%)	≤100 (90%)	Good quality
			≤2000 (95%)		Medium quality
			≥2000 (95%) 5%<n<33%		Poor quality
			≥2000 (95%) n>33,3%		Bad quality
Syria		0-100 (50%) 101-1000 (90%) 1001 and more			Good quality Just acceptable quality Unacceptable

Tunisia	500 (80%)	100 (80%)		100 (90%)	Unofficially the EU Directive for special purposes, e.g. blue flag programme
Turkey	1000 (100%)	200 (100%)			Officially the EU Directive
UNEP/WHO	50%<100 90%<1,000				

4.1.2 WHO Guidelines for safe recreational water environments

A WHO expert group on recreational waters agreed that the degree of convergence among the outcomes and findings of the principal epidemiological studies correlating recreational water quality with health effects provided a sufficiently solid basis from which to derive guideline values (WHO 1999). Between 1994 and 2003 WHO facilitated a series of expert meetings to design evidence-based guidelines for safe recreational water environments (WHO, 2003). A number of key outcomes resulted from this process:

- Derivation of dose-response curves from randomized healthy adult volunteer prospective epidemiological studies carried out by Kay *et al.,* (1994).
- Derivation of guideline values for microbial marine water quality (WHO, 2003) (Table 8)
- A harmonized approach to assessment of risk and risk management of recreational waters. This approach moves away from the reliance on numerical values of faecal indicator bacteria as the only compliance criterion to the use of qualitative ranking of faecal loading in recreational water environments, supported by direct measurement of faecal indices (WHO, 1999).
- An approach to estimate disease burden described as quantitative microbial risk assessment (QMRA). This approach requires identification of reference pathogens and estimation of their concentrations from sources or points of exposure followed by the application of pathogen dose-response relationships and an estimation of the probability of illness/infection (WHO, 2003).

It is undoubtedly the case that coastal bathing water quality can result in illness of varying severities. Whilst a potentially large disease burden associated with bathing water quality exists, there may be scope for significant reductions to be made. The move away from a reliance on a single guideline value advocated by WHO and the new EU bathing water Directive aims towards a system that provides for a comprehensive and flexible approach to the control of recreational water environments that better reflects health risks and provides enhanced scope for effective management intervention. Such an approach is enshrined in the approach known as the "Annapolis Protocol" (WHO, 1999), and involved an extended implementation of the WHO methodology to derive the new EU Guideline Values. The group's conclusions led to the development by WHO of its Guidelines for safe recreational water environments (WHO 2003), intended to be used as a basis for the development of international and national approaches to the control of hazards that may be encountered in such environments. The guideline values presented are not mandatory limits, but measures of the safety of a recreational water environment (Table 8). It is stated that the main reason for not promoting the adoption of international standards for such environments is the advantage provided by the adoption of a risk-benefit approach. In the specific case of recreational water use, development of such approaches not only concerns health risks and benefits, but inter-relates with other risks and benefits, especially those concerning environmental pollution / conservation as well as local and national economic and health benefits and well-being derived from recreational use of the water environment. It is similarly stated that this approach can often lead to the adoption of standards that are measurable, and can be implemented and enforced.

Table 8
WHO Guideline values for microbial quality of recreational water (WHO, 2003)

95th percentile value of intestinal enterococci/100 ml (rounded values)	Basis of derivation	Estimated risk per exposure
≤40 A	This range is below the NOAEL in most epidemiological studies.	**<1% GI illness risk** **<0.3% AFRI risk** The upper 95th percentile value of 40/100 ml relates to an average probability of less than one case of gastroenteritis in every 100 exposures. The AFRI burden would be negligible.
41–200 B	The 200/100 ml value is above the threshold of illness transmission reported in most epidemiological studies that have attempted to define a NOAEL or LOAEL for GI illness and AFRI.	**1–5% GI illness risk** **0.3–1.9% AFRI risk** The upper 95th percentile value of 200/100 ml relates to an average probability of one case of gastroenteritis in 20 exposures. The AFRI illness rate at this upper value would be less than 19 per 1000 exposures, or less than approximately 1 in 50 exposures.
201–500 C	This range represents a substantial elevation in the probability of all adverse health outcomes for which dose–response data are available.	**5–10% GI illness risk** **1.9–3.9% AFRI risk** This range of 95th percentiles represents a probability of 1 in 10 to 1 in 20 of gastroenteritis for a single exposure. Exposures in this category also suggest a risk of AFRI in the range of 19–39 per 1000 exposures, or a range of approximately 1 in 50 to 1 in 25 exposures.
>500 D	Above this level, there may be a significant risk of high levels of minor illness transmission.	**>10% GI illness risk** **>3.9% AFRI risk** There is a greater than 10% chance of gastroenteritis per single exposure. The AFRI illness rate at the 95th percentile point of >500/100 ml would be greater than 39 per 1000 exposures, or greater than approximately 1 in 25 exposures.

Notes:

1. Abbreviations used: A–D are the corresponding microbial water quality assessment categories (see section 4.6) used as part of the classification procedure (Table 4.12); AFRI = acute febrile respiratory illness; GI = gastrointestinal; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level.
2. The "exposure" in the key studies was a minimum of 10 min of swimming involving three head immersions. It is envisaged that this is equivalent to many immersion activities of similar duration, but it may underestimate risk for longer periods of water contact or for activities involving higher risks of water ingestion (see also note 8).
3. The "estimated risk" refers to the excess risk of illness (relative to a group of non-bathers) among a group of bathers who have been exposed to faecally contaminated recreational water under conditions similar to those in the key studies.
4. The functional form used in the dose–response curve assumes no further illness outside the range of the data (i.e., at concentrations above 158 intestinal enterococci/100 ml; see Box 4.3). Thus, the estimates of illness rate reported above this value are likely to be underestimates of the actual disease incidence attributable to recreational water exposure.
5. The estimated risks were derived from sewage-impacted marine waters. Different sources of pollution and more or less aggressive environments may modify the risks.
6. This table is derived from risk to healthy adult bathers exposed to marine waters in temperate north European waters.

The WHO Guidelines review 22 epidemiological studies, including six from the Mediterranean, and confirm that a sound public health basis for a causal relationship between faecal contamination of recreational water and adverse health outcomes amongst user groups ingesting contaminated water exists. It is also stated that the overall body of evidence from epidemiological studies indicates that mild to moderate self-limiting symptoms are reported among user groups ingesting faecally-contaminated water at frequencies that increase with exposure.

The guideline values for the microbiological quality of marine recreational waters, expressed as faecal streptococci levels per 100 ml, are given in Table 8 and related to the classification status of the water in Table 9. It is stated that (1) values given would produce protection of "healthy adult bathers" exposed to marine waters in temperate North European countries, (2) values do not relate to children, the elderly or immunocompromised who would have lower immunity and might require a greater degree of protection, and (3) epidemiological data on fresh waters or exposures other than bathing (*e.g.* high exposure activities such as surfing or white water canoeing) are currently inadequate to present a parallel analysis for defined reference risks. Thus a single guideline value is proposed *at this time* for all recreational uses of water because insufficient evidence exists at present to do otherwise. However, it is recommended that the severity and frequency of exposure encountered by special interest groups (such as body, board and wind-surfers, sub-aqua divers, canoeists and dinghy sailors) be taken into account.

Table 9
Example of a classification matrix for faecal pollution of recreational water environments (WHO, 2003)

		Microbial Water Quality Assessment Category (95th percentile intestinal enterococci/100 ml)				
		A ≤40	**B** 41-200	**C** 201-500	**D** >500	**Exceptional circumstances**
Sanitary Inspection Category (susceptibility to faecal influence)	**Very low**	Very good	Very good	Follow up	Follow up	Action
	Low	Very good	Good	Fair	Follow up	
	moderate	Good	Good	Fair	Poor	
	High	Good	Fair	Poor	Very poor	
	Very high	Follow up	Fair	Poor	Very poor	
Exceptional circumstances		Action				

4.2 EC Directive on bathing waters

A new EU law on bathing water quality was entered into force on 24 March 2006. The new Directive reflects EU policy to move away from Directives based primarily on achieving compliance, to Directives based on outcomes which allow a degree of subsidiarity. The new Bathing Water Directive is considerably more flexible than the existing Directive and is focused on protection of public health by a combination of planning, public information and standards. In particular, it describes new standards for indicator microorganisms in coastal and inland waters based on research which informed the recently published WHO Recreational Water Guidelines. There is also a requirement for a review process to take account of new research findings. It also strengthens rules first passed in 1976 by introducing a new "sufficient" water quality category and setting stricter standards for bacterial pollution in coastal and inland waters.

The new standards and associated monitoring requirements are reflected in four bathing water classifications which will be applied and published in all 25 EU Member States so that citizens are aware of bathing water quality wherever they visit. Member States must aim to achieve at least the minimum 'satisfactory' classification by 2015. The directive brings down the number of quality tests from nineteen to two, focusing on bacteria that most affect health: Escherichia coli and intestinal enterococchi will be classified into four categories ranging from "excellent" to "poor". Those classed as poor will have to be reviewed every two years compared with three and four years for those with a higher ranking. Beaches with a "poor" rating for five years running face a permanent ban. Standards for intestinal enterococchi in the "sufficient" category are 185 for coastal waters, measured at 90 percentiles. The values for the bacterium Escherichia coli are unchanged (Table 10).

The Directive also requires active management including public participation, provision of information to the public and the preparation of bathing water profiles (sanitary inspections) to identify all potential sources of pollution. There is provision for limited discounting of poor samples if management action, such as public warnings or beach closures, has been carried out. Member States will be required to display signs at registered bathing sites to indicate water quality. They must provide the public with findings of regular quality tests, made available through the internet.

Table 10
Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC

Parameter (cfu/100 ml)	Excellent quality	Good quality	Sufficient	Reference method of analysis
Intestinal enterococci	100*	200*	185**	ISO 7899-1 or ISO 7899-2
E. coli	250*	500*	500**	ISO 9308-3 or ISO 9308-1

*based on 95-percentile evaluation
**based on 90-percentile evaluation

5. ANALYSIS OF MEDITERRANEAN BATHING WATERS 1996-2005

The number of participating countries has grown from 13 to 20 in the time span indicated. Figure 2 shows the number of countries that submitted data for the analysis between 1996 and 2005. This compares very favourably with previous surveys undertaken. Table 11 gives details of the countries submitting data.

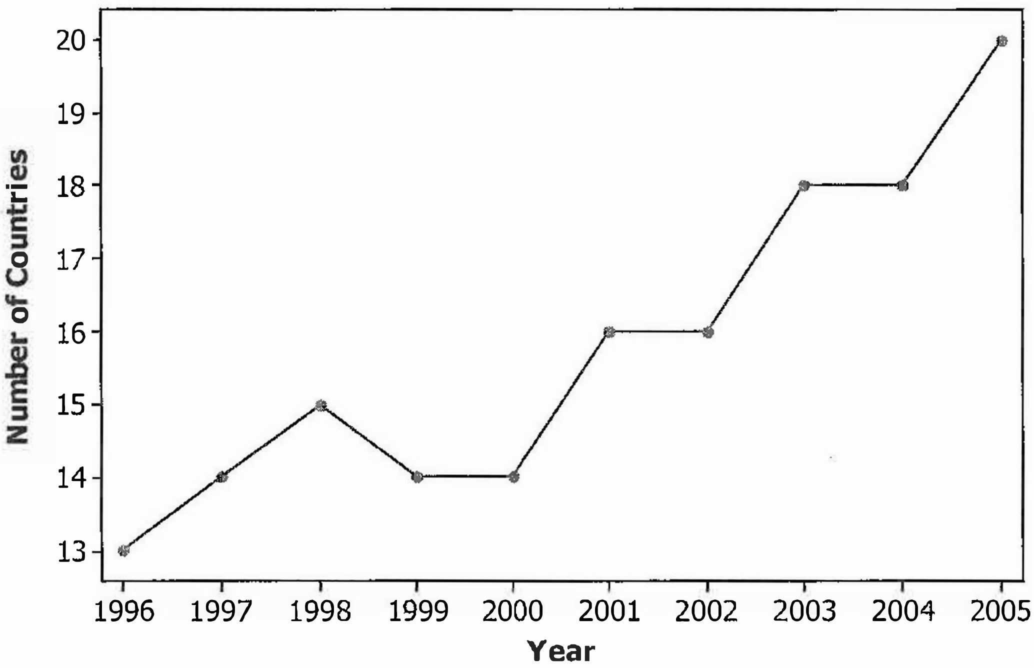

Figure 2. Number of countries submitting data (1996-2005)

Table 11
Countries submitting bathing water monitoring data (1996 – 2005)

year	Number of countries	Countries submitting monitoring data
1996	13	Algeria, Croatia, France, Greece, Israel, Italy, Libya, Malta, Monaco, Morocco, Slovenia, Spain, Tunisia
1997	14	Algeria, Croatia, France, Greece, Israel, Italy, Lebanon, Libya, Malta, Monaco, Morocco, Sloventia, Spain, Tunisia
1998	15	Algeria, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Malta, Monaco, Morocco Slovenia, Spain, Syria, Tunisia
1999	14	Algeria, Croatia, Cyprus, Greece, Israel, Italy, Lebanon, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia
2000	14	Algeria, Croatia, Cyprus, Greece, Israel, Italy, Lebanon, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia

2001	16	Algeria, Bosnia, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia
2002	16	Algeria, Bosnia, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia
2003	18	Albania, Algeria, Bosnia, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Libya, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia
2004	18	Albania, Algeria, Bosnia, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Libya, Malta, Monaco, Morocco, Slovenia, Spain, Syria, Tunisia
2005	20	Albania, Algeria, Bosnia, Croatia, Cyprus, France, Greece, Israel, Italy, Lebanon, Libya, Malta, Monaco, Morocco, Serbia and Montenegro, Slovenia, Spain, Syria, Tunisia, Turkey

With a slight downturn between 1998 and 1999, the overall number of sampling points has risen steadily indicating that more extensive areas of the Mediterranean are being monitored (Figure 3).

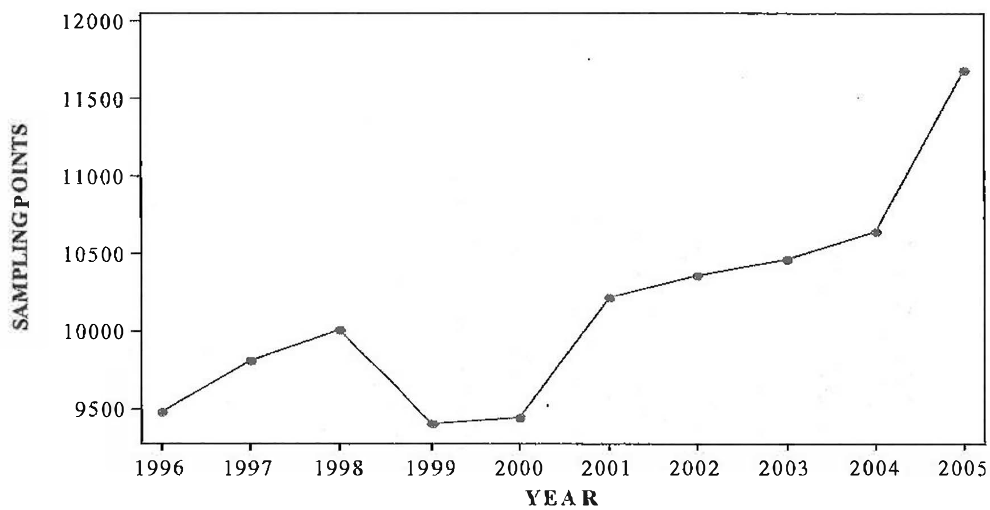

Figure 3. Number of sampling points per year (all countries) (1996-2005)

Table 12 and Figures 4-5 show the overall number of bathing waters in the Mediterranean conforming to and the number exceeding the national standards between the years 1996 and 2005. Whilst there has been a very slight overall increase (92.3% - 92.8%) in the percentage of bathing waters complying with national legislation the improvement has not been consistent over the years. Quality of those areas where monitoring takes place appears to have steadily increased until 2003 and then a slight worsening of quality are seen in 2004. A slight improvement is seen between 2004 and 2005. However, it should be noted that data from year to year is not strictly comparable due to the different countries participating in the survey from year to year, and the different legislative standards that each country complies with. It should also be noted that data only refers to waters that are officially monitored and there may be a number of bathing areas which are used for recreation that are not monitored.

Table 12
Summary of bathing water monitoring data per year complying and non-complying with the national legislation (1996 – 2005)

YEAR	Bathing waters CONFORMING	Bathing waters NOT CONFORMING	CONFORMING %	NOT CONFORMING %
1996	8747	734	92.3	7.7
1997	9036	769	92.2	7.8
1998	9390	619	93.8	6.2
1999	8873	534	94.3	5.7
2000	8818	620	93.4	6.6
2001	9617	593	94.2	5.8
2002	9745	608	94.1	5.9
2003	9887	549	94.5	5.3
2004	9803	834	92.2	7.8
2005	10842	839	92.8	7.2

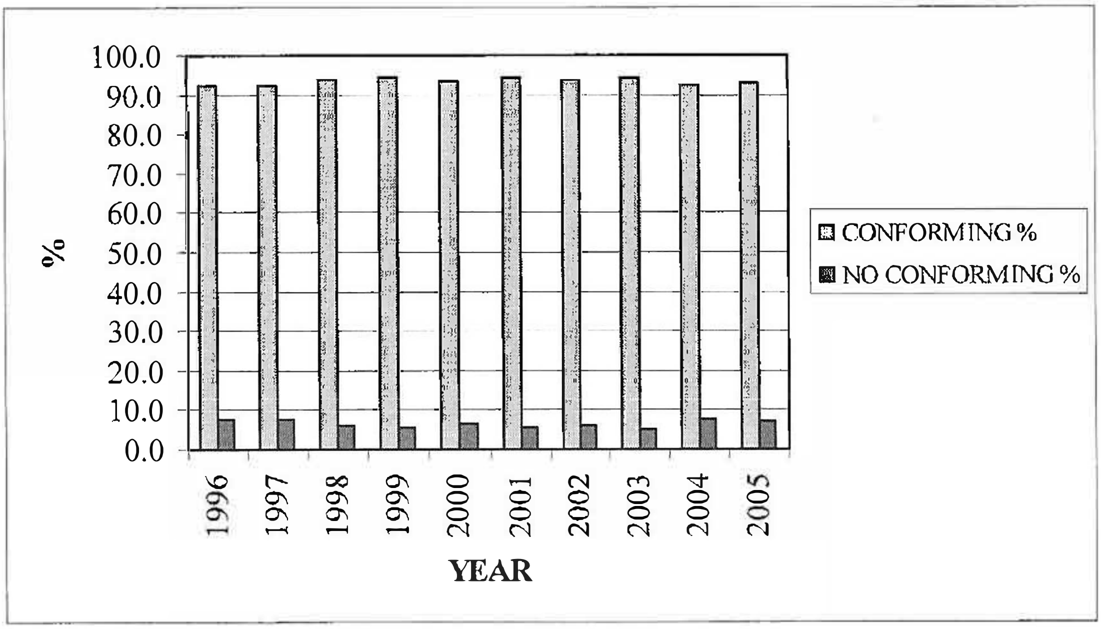

Figure 4. Percentage of bathing waters complying and not complying with national legislation per year (1996 – 2005)

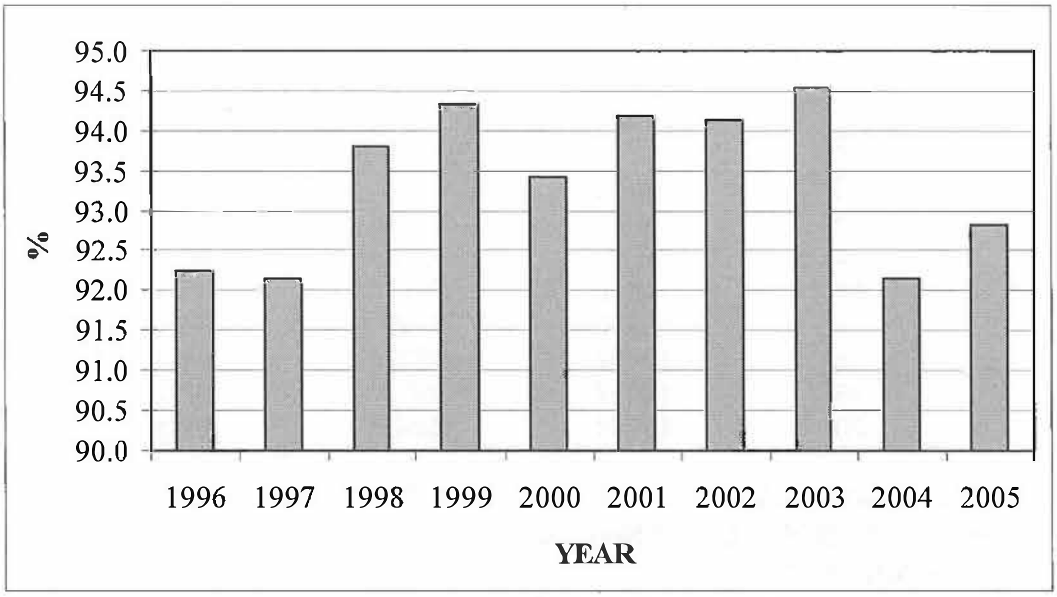

Figure 5. Percentage of bathing waters complying with national legislation per year (1996 – 2005)

Table 13 and Figures 6-8 summarises the data from the previous survey (utilising data from 1983 to 1995) and the current survey (1996-2005). This shows that although the overall quality has shown a slight decline in recent years (2003-2005), in general there has been an improvement both in the participation in the survey and in the quality of the Mediterranean since 1983. As in previous years there is a geographical imbalance in the distribution of the sampling points, the northern and western parts of the region submitting data from a greater number of sampling points than the east and the south.

Table 13
Summary of bathing water monitoring data per year (1983 – 2005)

Year	Number of countries submitting data	Number of Stations monitored	sampling points complying with national standards	% sampling points complying with national standards
1983	8	139	50	36,0
1984	9	183	85	46,4
1985	10	344	99	28,8
1986	10	352	191	54,3
1987	10	353	221	62,6
1988	10	354	227	64,1
1989	12	414	248	59,9
1990	10	376	263	69,9
1991	13	8169	7215	88,3
1992	9	8909	7831	87,9
1993	4	8799	7836	89.1
1994	4	9185	8261	89.9
1995	-	-	-	-
1996	13	9481	8747	92,3
1997	14	9805	9036	92,2

Year	Number of countries submitting data	Number of Stations monitored	sampling points complying with national standards	% sampling points complying with national standards
1998	15	10009	9390	93,8
1999	14	9407	8873	94,3
2000	14	9438	8818	93,4
2001	16	10210	9617	94,2
2002	16	10353	9745	94,1
2003	18	10457	9887	94,5
2004	18	10637	9803	92,2
2005	20	11681	10842	92,8

- 1983 – 1990 Non E.C.
- 1991 – 1992 Both E.C. and Non E.C.
- 1993 – 1994 E.C.
- 1983 – 1991: Former Yugoslavia x 4

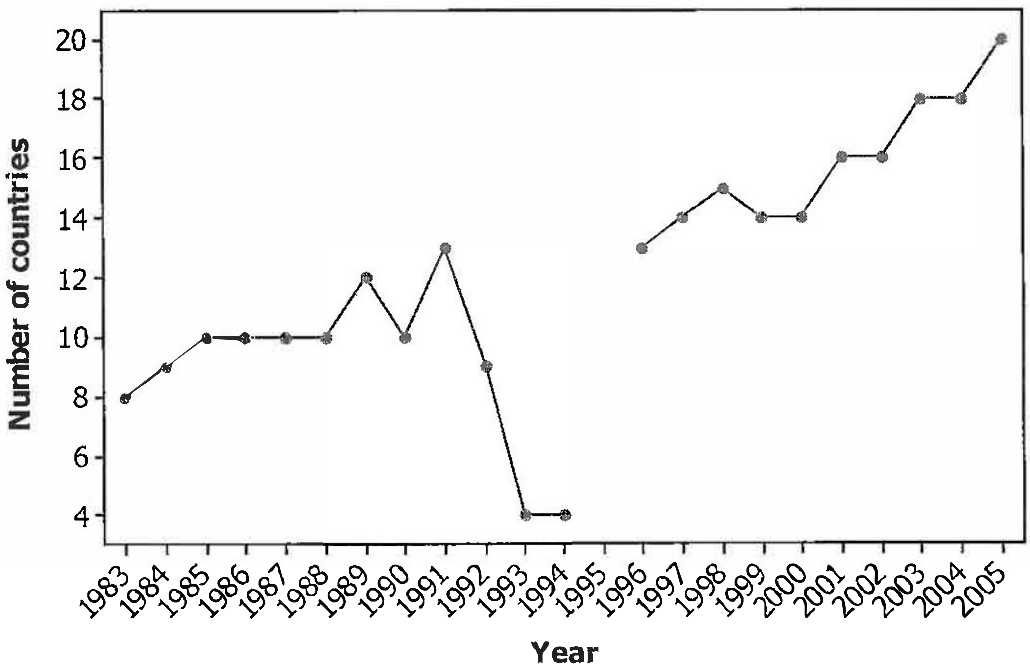

Figure 6. Time series plot of number of countries submitting data (1983 -2005)

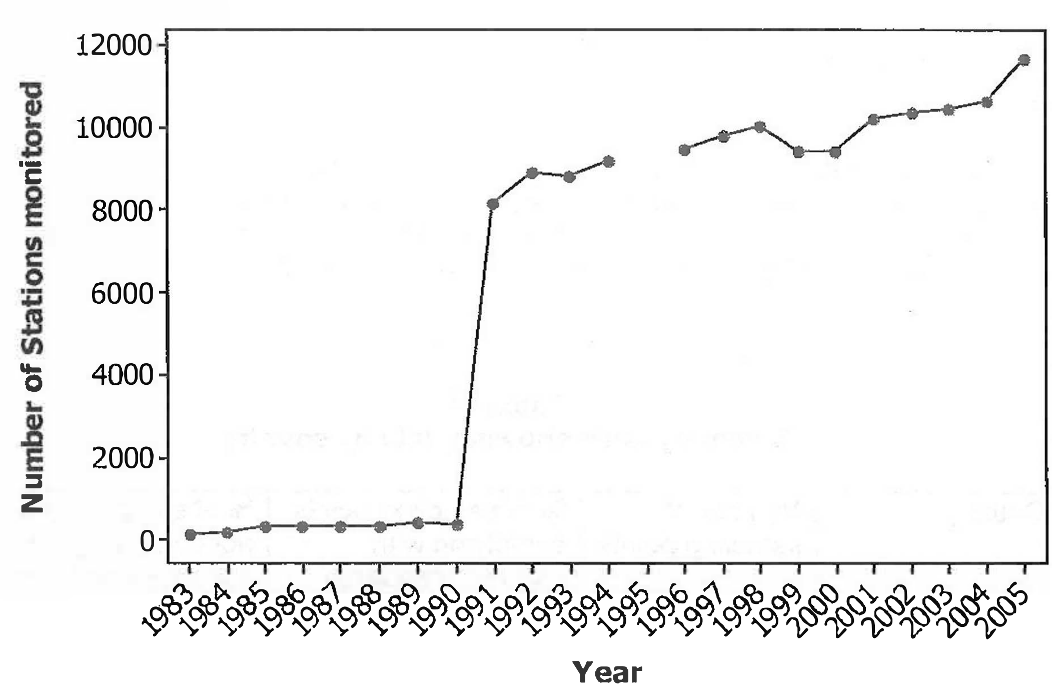

Figure 7. Time series Plot of Number of Stations monitored (1983 – 2005)

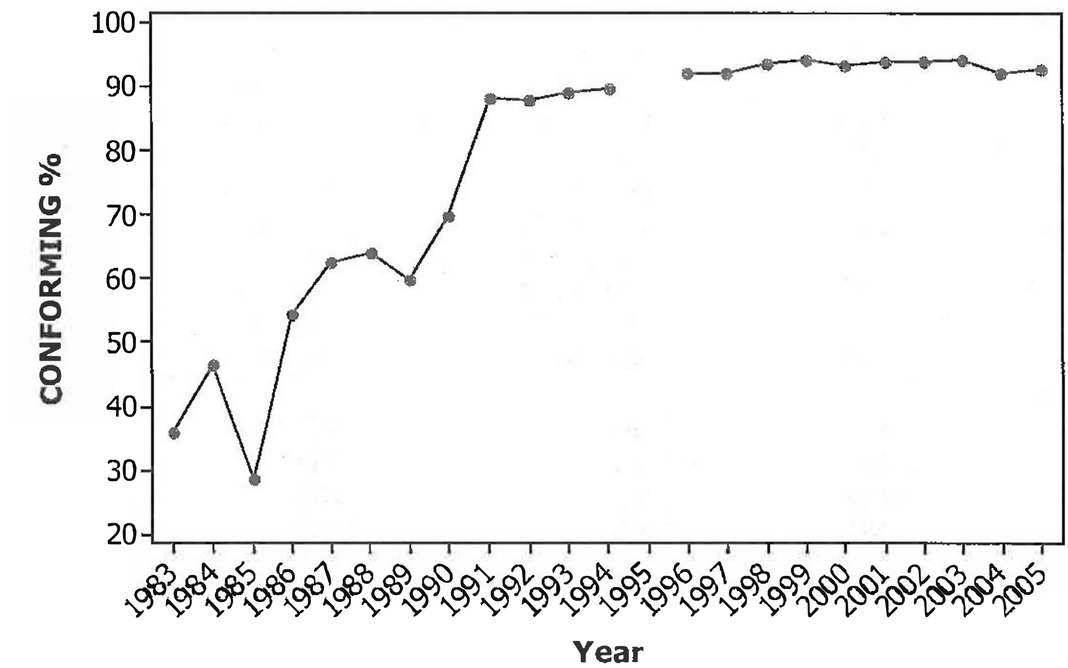

Figure 8. Percentage of bathing beaches conforming per year for all countries participating (1983 – 2005)

Table 14 shows the breakdown of the data by country. In all cases except Malta, Cyprus, Slovenia, Tunisia and Monaco, the number of sampling points has increased between 1996 and 2005 reflecting the overall increased monitoring occurring around the Mediterranean bathing areas.

Although there is no real trend evident during the sampling period it can be seen that, encouragingly, 50% of the countries submitting data for 2005 achieved over 90% compliance with national standards for bathing water quality. Malta, Cyprus, Greece, Turkey and Monaco all achieved 100% compliance with their national standards by 2005.

Table 14
Summary table showing data by country

Country	Number of sampling points	% of sampling points complying with national standards	% of sampling points not complying with national standards
ALBANIA			
1996	ND	ND	ND
1997	ND	ND	ND
1998	ND	ND	ND
1999	ND	ND	ND
2000	ND	ND	ND
2001	ND	ND	ND
2002	ND	ND	ND
2003	50	33 (66)	17 (34)
2004	31	16 (51.6)	15 (48.4)
2005	67	31(46.2)	36 (53.8)
ALGERIA			
1996	90	50 (55.5)	40 (44.5)
1997	149	70 (47.0)	79 (53.0)
1998	120	89 (74.2)	31 (25.8)
1999	100	97 (97.0)	3 (3.0)
2000	150	80 (53.3)	70 (46.7)
2001	263	152 (57.7)	111 (42.3)
2002	271	201 (74.2)	70 (25.8)
2003	272	222 (81.6)	50 (18.4)
2004	278	80 (28.8)	198 (71.2)
2005	292	200 (68.5)	92 (31.5)
BOSNIA			
1996	ND	ND	ND
1997	ND	ND	ND
1998	ND	ND	ND
1999	ND	ND	ND
2000	ND	ND	ND
2001	8	6 (75.0)	2 (25.0)
2002	12	12 (100)	0 (0)
2003	12	12 (100)	0 (0)
2004	12	5 (41.6)	7 (58.4)
2005	12	5 (41.6)	7 (58.4)

Country	Number of sampling points	% of sampling points complying with national standards	% of sampling points not complying with national standards
CROATIA			
1996	732	690 (94.2)	42 (5.8)
1997	831	812 (97.7)	19 (2.3)
1998	769	752 (97.8)	17 (2.2)
1999	803	793 (98.7)	10 (1.3)
2000	702	621 (88.5)	81 (11.5)
2001	728	653 (89.7)	75 (10.3)
2002	770	702 (91.2)	68 (8.8)
2003	841	796 (94.6)	45 (5.4)
2004	846	806 (95.3)	40 (4.7)
2005	851	808 (94.9)	43 (5.1)
CYPRUS			
1996	ND	ND	ND
1997	ND	ND	ND
1998	165	164 (99.3)	1 (0.7)
1999	165	165 (100)	0 (0.0)
2000	153	153 (100)	0 (0.0)
2001	105	105 (100)	0 (0.0)
2002	109	109 (100)	0 (0.0)
2003	121	121 (100)	0 (0.0)
2004	112	112 (100)	0 (0.0)
2005	100	100 (100)	0 (0.0)
FRANCE			
1996	670	636 (95.0)	34 (5.0)
1997	636	557 (87.6)	79 (2.4)
1998	669	662 (99.0)	7 (1.0)
1999	ND	ND	ND
2000	ND	ND	ND
2001	672	656 (97.6)	16 (2.4)
2002	708	684 (96.6)	24 (3.4)
2003	670	655 (97.8)	15 (2.2)
2004	678	660 (97.3)	18 (2.7)
2005	685	663 (97.0)	22 (3.0)
GREECE			
1996	1690	1666 (98.6)	24 (1.4)
1997	1701	1674 (98.4)	27 (1.6)
1998	1733	1710 (98.7)	23 (1.3)
1999	1816	1795 (98.8)	21 (1.2)
2000	1858	1835 (98.8)	23 (1.2)
2001	1887	1875 (99.4)	12 (0.6)
2002	1914	1912 (99.9)	2 (0.1)
2003	1933	1931 (99.9)	2 (0.1)
2004	1965	1964 (99.9)	1 (0.1)
2005	2006	2006 (100)	0 (0.0)
ISRAEL			
1996	95	94 (98.9)	1 (1.1)
1997	95	91 (95.8)	4 (4.2)
1998	113	109 (96.5)	4 (3.5)
1999	112	108 (96.4)	4 (3.6)
2000	113	109 (96.5)	4 (3.5)
2001	113	109 (96.5)	4 (3.5)

Country	Number of sampling points	% of sampling points complying with national standards	% of sampling points not complying with national standards
2002	107	103 (96.3)	4 (3.7)
2003	109	105 (96.3)	4 (3.7)
2004	114	110 (96.5)	4 (3.6)
2005	106	104 (98.1)	2 (1.9)
ITALY			
1996	4688	4333 (92.4)	355 (7.6)
1997	4836	4576 (94.6)	260 (5.4)
1998	4868	4593 (94.4)	275 (5.6)
1999	4811	4623 (96.1)	188 (3.9)
2000	4819	4607 (95.6)	212 (4.4)
2001	4824	4662 (96.6)	162 (3.4)
2002	4850	4663 (96.1)	187 (3.9)
2003	4853	4630 (95.4)	223 (4.6)
2004	4884	4627 (94.7)	257 (5.3)
2005	4919	4616 (93.8)	303 (6.2)
LEBANON			
1996	ND	ND	ND
1997	13	11 (84.6)	2 (15.4)
1998	13	11 (84.6)	2 (15.4)
1999	10	8 (80.0)	2 (20.0)
2000	11	8 (73.0)	3 (27.0)
2001	15	12 (80.0)	3 (20.0)
2002	16	12 (75.0)	4 (25.0)
2003	17	13 (76.4)	4 (23.6)
2004	17	15 (88.2)	2 (11.8)
2005	17	12 (70.5)	5 (29.5)
LIBYA	16	11 (68.7)	5 (31.3)
1996	16	16 (100)	0 (0.0)
1997	ND	ND	ND
1998	ND	ND	ND
1999	ND	ND	ND
2000	ND	ND	ND
2001	ND	ND	ND
2002	16	13 (81.3)	3 (18.7)
2003	ND	ND	ND
2004	30	27 (90.0)	3 (10.0)
2005			
MALTA			
1996	92	90 (97.8)	2 (2.2)
1997	92	89 (96.8)	3 (3.2)
1998	93	87 (93.5)	6 (6.5)
1999	93	89 (95.7)	4 (4.3)
2000	96	94 (98.0)	2 (2.0)
2001	87	87 (100)	0 (0.0)
2002	87	87 (100)	0 (0.0)
2003	87	87 (100)	0 (0.0)
2004	87	87 (100)	0 (0.0)
2005	87	87 (100)	0 (0.0)
MONACO			
1996	10	10 (100)	0 (0)
1997	11	11 (100)	0 (0)

Country	Number of sampling points	% of sampling points complying with national standards	% of sampling points not complying with national standards
1998	11	11 (100)	0 (0)
1999	10	10 (100)	0 (0)
2000	10	10 (100)	0 (0)
2001	6	6 (100)	0 (0)
2002	6	6 (100)	0 (0)
2003	6	6 (100)	0 (0)
2004	6	6 (100)	0 (0)
2005	6	6 (100)	0 (0)
MONTENEGRO			
1996	ND	ND	ND
1997	ND	ND	ND
1998	ND	ND	ND
1999	ND	ND	ND
2000	ND	ND	ND
2001	ND	ND	ND
2002	ND	ND	ND
2003	ND	ND	ND
2004	75	15 (20)	60 (80)
2005	70	20 (28.6)	50 (71.4)
MOROCCO			
1996	12	8 (66.7)	4 (33.3)
1997	12	8 (66.7)	4 (33.3)
1998	12	8 (66.7)	4 (33.3)
1999	17	12 (70.6)	5 (29.4)
2000	19	15 (79.0)	4 (21.0)
2001	19	14 (73.7)	5 (26.3)
2002	24	20 (83.3)	4 (16.7)
2003	24	22 (91.7)	2 (8.3)
2004	28	25 (89.3)	3 (10.7)
2005	71	57 (80.3)	14 (19.7)
SLOVENIA			
1996	32	12 (37.5)	20 (62.5)
1997	32	24 (75.0)	8 (25.0)
1998	32	8 (25.0)	24 (75.0)
1999	32	27 (84.5)	5 (15.4)
2000	32	31 (96.9)	1 (3.1)
2001	32	32 (100)	0 (0.0)
2002	20	20 (100)	0 (0.0)
2003	21	ND	ND
2004	19	17 (89.5)	2 (10.5)
2005	19	18 (94.7)	1 (5.3)
SPAIN			
1996	839	828 (98.7)	11 (1.3)
1997	866	855 (98.7)	11 (1.3)
1998	869	852 (98.4)	17 (1.6)
1999	896	874 (97.5)	22 (2.5)
2000	905	887 (98.0)	18 (0.2)
2001	913	893 (97.8)	20 (2.2)
2002	916	903 (98.6)	13 (1.4)
2003	914	898 (98.2)	16 (1.8)
2004	934	926 (99.1)	8 (0.9)

Country	Number of sampling points	% of sampling points complying with national standards	% of sampling points not complying with national standards
2005	938	931 (99.2)	7 (0.8)
SYRIA			
1996	ND	ND	ND
1997	ND	ND	ND
1998	27	20 (74.0)	7 (26.0)
1999	27	20 (74.0)	7 (26.0)
2000	24	19 (79.2)	5 (20.8)
2001	26	21 (81.0)	5 (19.0)
2002	27	22 (81.5)	5 (18.5)
2003	27	22 (81.5)	5 (18.5)
2004	28	23 (82.1)	5 (17.9)
2005	28	23 (82.1)	5 (17.9)
TURKEY			
1996	ND	ND	ND
1997	ND	ND	ND
1998	ND	ND	ND
1999	ND	ND	ND
2000	ND	ND	ND
2001	ND	ND	ND
2002	ND	ND	ND
2003	ND	ND	ND
2004	ND	ND	ND
2005	871	871 (100)	0
TUNISIA			
1996	515	319 (62)	196 (38)
1997	515	242 (47)	273 (53)
1998	515	314 (61)	201 (39)
1999	515	252 (49)	263 (51)
2000	546	349 (64)	197 (36)
2001	512	334 (65)	178 (35)
2002	516	289 (56)	227 (44)
2003	484	321 (66)	163 (34)
2004	523	309 (59)	214 (41)
2005	506	259 (51)	247 (49)

6. SHELLFISH-GROWING WATERS AND THEIR MICROBIOLOGICAL QUALITY

Many factors influence the suitability of coastal areas for growing and harvesting shellfish, and none is more vital than clean water. Human habitation has had a dramatic effect on the condition of coastal habitats and resources. A primary concern in shellfish growing areas—which are generally located in the intertidal and shallow subtidal coastal zone — is contamination from human sewage and animal wastes and the related health risks associated with the consumption of contaminated shellfish.

The main sources of faecal pollution include municipal sewage systems, on-site sewage systems, stormwater runoff, marinas and boaters, farm animals, pets and wildlife. As is the case with other coastal habitats, the condition and classification of shellfish growing areas generally correlate with human population densities and land uses in adjacent shorelines and uplands. Rural watersheds with limited development and intact land cover are best suited to shellfish harvesting, and more developed watersheds are less so. Population growth and development are rapidly changing the landscape of the Mediterranean and, in turn, are placing greater pressure on shellfish harvesting and other valued uses and functions of the coastal environment. When left unchecked, the process of urbanization—defined as the transformation of natural landscapes to built environments—can leave coastal areas permanently unfit for the harvest and consumption of shellfish.

There are different water quality conditions associated with different types, patterns, and densities of coastal development, but our limited understanding of these relationships hampers our efforts to effectively manage land uses and control pollution to permanently safeguard water quality for shellfish harvesting in the Mediterranean.

Estuaries support many functions and uses, and no use is more dependent on clean water, more vulnerable to the effects of pollution and the transmission of disease, than the harvest and consumption of shellfish. Oysters, clams, and other bivalve molluscan shellfish feed by filtering plankton and other particles from the surrounding water and sediments and in the process can accumulate disease-causing microorganisms (pathogens) and other contaminants that may be present in the nearshore environment.

Most health risks associated with the consumption of shellfish and other seafoods are attributed to the environment where the products are grown and harvested. To address these risks all commercial shellfish growing areas are monitored and classified under each country's national shellfish monitoring project. The main assessment tool of each national project is a comprehensive sanitary survey. Surveys should be conducted on a regular basis for all commercial growing areas and consist of ongoing water quality monitoring (principally measurements of *E. coli*), pollution source investigations, and meteorological and hydrographic evaluations. Because of the difficulty and expense associated with the direct detection of pathogens in water or shellfish, faecal coliform bacteria and *E. coli* are widely used as indicator organisms to signal the possible presence of faeces and pathogenic organisms.

While bacterial indicators have proved to be useful in helping to assess the sanitary condition of shellfish growing areas, there is growing recognition that they do not reliably predict the occurrence and survival of enteric viruses and other pathogens in the marine environment (Bosch, 1998; Goyal *et al.,* 1984; Hernroth *et al.,* 2002; Jiang *et al.,* 2001; Lees, 2000; Lilja and Glasoe, 1993; NRC, 2004; Noble *et al.,* 2003a; Noble and Furmen, 2001; Schroeder *et al.,* 2002; USEPA, 2001c; Wetz *et al.,* 2004; Vasconcelos, 2001). Other factors further complicate the indicator system and the task of accurately gauging growing-area conditions and related health risks. These include variability in sampling procedures as well as unique geographic, hydrographic, and anthropogenic factors such as climate and weather patterns, circulation patterns and water properties, watershed hydrology and geology, land cover and land use patterns, pollution sources and management practices, and population densities and patterns (Bennett *et al.,* 2000; Boehm *et al.,* 2002; De Luca-Abbott *et al.,* 2000; Henrickson *et al.,* 2001; Leecaster and Weisberg, 2001; Lipp *et al.,* 2001b; Noble *et al.,* 2003b, 2001; Rose *et al.,* 2001a, 2001b; Smith *et al.,* 2001).

6.1 Effects of urbanization on watershed hydrology and water quality

Aquatic habitats are integral parts of the natural landscape, shaped and defined by many interacting physical, chemical, and biological processes over time and space (Hynes, 1975; Karr, 1998; Naiman *et al.,* 1992; Spence *et al.,* 1996; Turner, 1994). Numerous studies have shown that human modification of the natural landscape has a direct and significant effect on the condition of aquatic systems, including both stream systems (Bolstad and Swank, 1997; Booth, 1991; Booth and Jackson, 1997; Bunn and Arthington, 2002; Hunsaker and Levine, 1995; May *et al.,* 2000; Nelson and Booth, 2002; Paul and Meyer, 2001; Poff *et* *al.,* 1997; Roth *et al.,* 1996; Snyder *et al.,* 2003; Wang *et al.,* 2001; Wear *et al.,* 1998) and nearshore marine systems (Bay *et al.,* 2003, 1999; Bowen and Valiela, 2001; Breitburg *et al.,* 2003; Dojiri *et al.,* 2003; Holland, 2000; Holland *et al.,* 2004, 1998; Hopkinson and Vallino, 1995; Lerberg *et al.,* 2000; Mallin *et al.,* 2001, 2000a, 2000b; Mallin and Lewitus, 2004; Sanger *et al.,* 1999a, 1999b; Valiela *et al.,* 1992; Van Dolah *et al.,* 2000; Vernberg *et al.,* 1999, 1996, 1992; Vernberg and Vernberg, 2001). Primary impacts include the fragmentation and loss of habitats as well as the degradation of water resources and water quality (USEPA, 2001b). For shellfish resources, both types of impacts are relevant and important and are best explained in a landscape context.

Microorganisms are discharged to shellfish growing areas from a variety of pollution sources along three main pathways: (1) direct discharges from sewage outfalls, boaters, marine mammals, and other sources; (2) subsurface flows from such sources as shoreline on-site sewage systems; and (3) overland flows in the form of stormwater runoff, stream flows and other surface runoff. These sources and pathways are determined by a variety of human activities and land uses that tend to exert a progressively greater influence on the landscape and environmental conditions as development intensifies over time. Although bacterial loadings and shellfish impacts generally correlate with the intensity of adjacent land uses, it is important to note that shellfish growing areas can be contaminated and closed in areas with limited development if raw sewage or animal wastes are being discharged to the waters. By its very nature, nonpoint source pollution presents risks that must be addressed in all shellfish areas, regardless of the degree of development. The transformation of landscapes from rural to urban land uses simply compounds the problem.

Studies have also documented high levels of selected pathogens in stormwater discharges (Burton and Pitt, 2002). Faecal coliform concentrations are influenced by such factors as rainfall and drainage area characteristics, including land uses, faecal pollution sources, and runoff potential of different surfaces and landscapes (Pitt, 2000). Illustrating the importance of source-area characteristics, Pitt *et al.* (2004) reported average faecal coliform concentrations of 7,750 for residential areas, 4,500 for commercial areas, and 2,500 for industrial areas. Pollution sources that can potentially contribute to stormwater contamination include cross connections with sewage lines, failing on-site sewage systems, pet and other animal wastes, and even bacterial growth within the drainage system itself. None of the potential sources is benign and the cumulative loadings can be immense. Dog faeces, for example, has been estimated to contain 23 million faecal coliform bacteria per gram (Schueler, 2000c) and pet wastes have been identified as key pollution sources in many shellfish contamination studies (Kelsey *et al.,* 2003, 2004; Mallin *et al.,* 2001; Van Dolah *et al.,* 2000; White *et al.,* 2000). More broadly, numerous other studies have identified stormwater runoff and stream flows associated with rainfall events as major sources of coastal microbial pollution (Ackerman and Weisberg, 2003; De Luca-Abbott *et al.,* 2000; Dwight *et al.,* 2002; Eisele *et al.,* 2001; Lipp *et al.,* 2001b; Marchman, 2000; Noble *et al.,* 2000a, 2000b).

6.2 Classification of shellfish-growing waters

Classification status of shellfish waters is based on sanitary surveys of water quality and shoreline surveys of pollution sources as well as on routine monitoring of water quality. In the United States, individual growing areas are classified either as approved for harvest or as one of harvest-limited categories: (1) conditionally approved, (2) restricted, (3) conditionally restricted, or (4) prohibited. All identified growing waters must be classified as prohibited unless sanitary surveys indicate that water quality meets specific national standards for the other categories. Harvesting is permissible in approved areas year-round. Shellfish harvested from restricted areas must be relayed to approved waters or to "depuration" facilities for a designated period of time to reduce their levels of bacteria and viruses before they are processed for human consumption.

The criteria for classification (EU Regulation 854/2004) of shellfish growing harvesting areas as given in European legislation are outlined in Table 15.

Table 15
Criteria for the classification of bivalve mollusc harvesting areas under Regulation (EC) No 854/2004 and, by cross-reference, in the Council Regulation on microbiological criteria for foodstuffs

Class	Microbiological Standard	Post harvest treatment
A	Live bivalve molluscs from these area must not exceed 230 MPN *E.coli* per 100g of flesh and intravalvular liquid	None
B	Live bivalve molluscs from these area must not exceed the limits of a five tube, three dilution MPN test of 4600 *E.coli* per 100g of flesh and intravalvular liquid	Purification, relaying or cooking by an approved method
C	Live bivalve molluscs from these area must not exceed the limits of a five tube, three dilution MPN test of 46000 *E. coli* per 100 g of flesh and intravalvular liquid	Relaying for a long period or cooking by an approved method
Prohibited	Live bivalve molluscs from these area must not exceed 230 MPN *E. coli* per 100 g of flesh and intravalvular liquid	Harvesting is not permitted

In Mediterranean countries, individual growing areas are classified according to European standards for those countries belonging to the EU. The rest of the Mediterranean countries follow national guidelines or the European standards.

Conditionally approved and conditionally restricted categories are voluntary; countries can use these categories when a predictable pollution event such as seasonal population, heavy rainfall or fluctuating discharges from local sewage plants affect the suitability of an area for harvest. Harvests from waters in these categories typically require substantial state resources for issuing permits, monitoring water quality, creating a management plan, and supervising harvest and transportation. Unfortunately, some potentially productive growing areas remain prohibited for harvest because of inadequate state resources to conduct the requisite sanitary surveys.

The primary basis for harvest restrictions is the concentration of faecal coliform bacteria associated with human sewage and with organic wastes from livestock and wildlife. Other classification factors include proximity to known point and non-point sources of pollution, weather (e.g., heavy rainfall that temporarily introduces pollutants), tides, circulation and prevailing winds. In some cases, monitoring also includes toxic industrial contaminants, such as heavy metals, and marine biotoxins associated with coastal "blooms" of certain planktonic species – so-called "red tides." Some scientists and fishermen question the use of faecal coliform concentrations for indexing shellfish growing areas, particularly in waters that receive chlorine-disinfected effluents from wastewater treatment plants (Rippey, 1994).

Although chlorine is generally effective in inactivating most bacterial species, including bacterial pathogens, it is less effective against enteric viral pathogens found in sewage, such as the common Noroviruses associated with minor intestinal distress and related problems.

Most waterborne pathogens originate in human and animal faeces and include a wide variety of viruses, bacteria, and protozoa (Rose *et al.,* 1999). The transmission of viral disease is a key health concern associated with the consumption of shellfish. All of the known pathogenic viruses that present a significant public health threat in the marine environment are transmitted via the faecal-oral route and are known collectively as enteric viruses (Griffin *et al.* 2003). Lees (2000) points out that, of the many types of seafood, "only the bivalve molluscan shellfish have consistently proven to be an effective vehicle for the transmission of viral disease". Noroviruses and Hepatitis A virus are most commonly implicated in shellfish-related disease outbreaks (Bosch, 1998; Dadswel, 1993; Griffin *et al.,* 2003; Lees, 2000; Lipp and Rose, 1997; NRC, 1999; Richards, 1987; Sair *et al.,* 2002; Stelma and McCabe, 1992; Vasconcelos, 2001).

Because of the difficulty and expense associated with the direct detection of pathogens, faecal coliform bacteria are widely used as indicator organisms to signal the possible presence of faeces and pathogenic organisms. While bacterial indicators have proven useful in helping to assess the sanitary condition of shellfish growing areas, there is growing recognition that they do not reliably predict the occurrence and survival of enteric viruses and other pathogens in the marine environment (Bosch, 1998; Goyal *et al.,* 1984; Hernroth *et al.,* 2002; Jiang *et al.,* 2001; Lees, 2000; Lipp and Rose, 1997; NRC, 1999, 2004; Noble *et al.,* 2003a; Noble and Furman, 2001; Schroeder *et al.,* 2002; USEPA, 2001c; Wetz *et al.*, 2004; Vasconcelos, 2001). Other factors further complicate the indicator system and the task of accurately gauging growing-area conditions and related health risks. These include variability in sampling procedures as well as unique geographic, hydrographic, and anthropogenic factors such as climate and weather patterns, circulation patterns and water properties, watershed hydrology and geology, land cover and land use patterns, pollution sources and management practices, and population densities and patterns (Bennett *et al.,* 2000; Boehm *et al.,* 2002; De Luca-Abbott *et al.,* 2000; Henrickson *et al.,* 2001; Lester and Weisberg, 2001; Lipp *et al.*, 2001b; Noble *et al.,* 2003b; Rose *et al.*, 2001a, 2001b; Smith *et al.,* 2001). Any discussion of the relationship between coastal development and microbial contamination of shellfish growing areas must acknowledge and account for these factors and uncertainties (Table 16).

Table 16
Sources of faecal contamination of bivalve mollusc harvesting areas

Source	Level of risk to Public Health
a. Point Source Discharges	
Private/municipal sewage plant	Most significant risk because of diverse contributing population and volume dependent on various factors including volume of sewage, type of treatment and plant performance
Industrial waste resources (meat processing plants)	Significant risk if wastes involve pathogens capable of causing human disease or chemicals which can be accumulated; important primarily because of volume of waters
Combined sewer overflows	Significant risk because of untreated human waste contribution and volume

Septic tanks/soakaways	Low risk because of small volumes. May be significant local risk if not operating properly.
Animal feedlots/poultry houses	Potential human risk because of large aggregation of animals and ability of some domestic animals to transmit human diseases
b. Non point source Discharges	
Waste discharges from boats	Potential human risk due to possible intermittent discharge of small quantities of raw sewage
Storm drains, street runoff	Potential risk because human sewage contamination may be present; risk significantly less than combined sewers
Rural land with domestic animals	Significantly less risk than direct human sources
Nature reserve, forest, marsh, etc. (dominated by wild animals and birds)	Significantly less risk than human sources on present evidence

6.3 Legislation for international provisions for shellfish areas

A proposed draft code of hygiene practice for molluscan shellfish was prepared by the Codex Alimentarius Commission (1978). Appendix III to the draft code provided general recommendations on environmental sanitation, including the classification, control and re-classification of shellfish-growing areas. The situation prevailing at the time was that successful shellfish control programmes had been in operation in a number of countries for many years, using a wide range of bacteriological standards and methods, but at the same time, it was virtually impossible to reach agreement on any specific set of standards and methods (UNEP/WHO, 1985). The EU network of reference laboratories for monitoring bacteriological and viral contamination of bivalve molluscs was established under council Decision 99/313/EC. This designated CEFAS as the Community Reference laboratory and obliged Member States to designate National Reference Laboratories (NRLs).

6.3.1 Common Mediterranean standards and EC standards

Council Directive 79/923/EEC (EC, 1979) was introduced to protect and improve the quality of shellfish waters within the Community, and was applicable to the four Mediterranean Member States (France, Greece, Italy and Spain). The aim of defining quality objectives for shellfish waters is to protect the development of shellfish populations from the principal sources of pollution. The Directive stresses that it cannot, by itself, ensure protection of consumers of shellfish products, and that it is therefore necessary to take other measures to this effect. With this in mind, the Council adopted Directive 91/492/EEC, which lays down the health conditions for the production and the placing on the market of live bivalve molluscs. Unlike the case with bathing waters, the two international agreements on shellfish waters covering the Mediterranean (the 1979 EC Directive, which applies now directly to seven countries and serves as a model for others, and the 1987 common Mediterranean measure, which applies to all) are practically identical in so far as the microbiological components are concerned. The recent European legislation concerned with the quality of the shellfish as well as the shellfish growing water in Europe is the following:

- ➢ REGULATION (EC) No 853/2004 of the European Parliament and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin.
- ➢ REGULATION (EC) No 852/2004 of the European Parliament and of the council of 29 April 2004 on the hygiene of foodstuffs

- REGULATION (EC) No 854/2004 of the European Parliament and of the council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption
- REGULATION (EC) No 882/2004 of the European Parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
- COMMISSION REGULATION (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organization of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004
- COMMISSION REGULATION (EC) No 2076/2005 of 5 December 2005 laying down transitional arrangements for the implementation of Regulations (EC) No 853/2004, (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004
- COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

Numerous problems contribute to shellfish-associated infections. These problems regard handling the growth beds and storing, processing, labelling, and shipping the product—as well as lack of education. Both the United States and the European Union have recently established legislative standards to reduce the risk of contaminated shellfish reaching restaurants. The standards are centred on the detection of coliforms and *E. coli* in the United States, the standards focus on the growing waters of shellfish, and in the European Union, the standards focus on the number of microorganisms per 100 g of shellfish flesh. The fact that outbreaks of infection continue to occur despite apparently adequate control measures highlights the role of viruses, especially Noroviruses (NLVs). In fact, the published opinion of the scientific committee of the European Union on NLVs has recommended including NLVs in the communicable diseases surveillance network and assuring the implementation of safe food-handling measures. In addition, efforts have been initiated to educate consumers, restaurant proprietors, and physicians about the hazards of eating raw shellfish.

In the EU the responsibility for developing and applying official monitoring programmes lies with the competent authority and the monitoring requirements are given in Annex II of Regulation 854/2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption.

6.4 Existing national provisions for shellfish areas

The salient features of national legislation and related measures concerning the quality of shellfish waters and shellfish in the various Mediterranean countries are summarized in the following paragraphs. As in the case of recreational waters, the picture is not quite complete, as information from some countries was not made available by the time of finalisation of this document.

6.4.1 Albania

Approximately 58% of the Albanian population lives in the coastal areas along the Adriatic and the Ionian Seas. After 1991, most large Albanian industries (e.g. mineral production and processing, pesticides, fertilizers, chemicals, plastics, paper, food and textiles) were obliged to close down from these areas. This left stockpiles with obsolete hazardous substances as well as contaminated land. The main contamination problems are stockpiles of obsolete chemicals, untreated urban wastewater and solid wastes. Discharge of untreated urban wastewater, beach erosion and illegal construction on the coastline are seen at Vlora Bay, Porto Romano Bay, Durres Bay, Saranda Bay, Kune-Vaini lagoon, Drini River mouth (at the city of Lezhe), the Fieri district (on the Semani River), Karabasta lagoon and Divjaka Beach.

Standards for shellfish-growing water quality in Albania are established by a Ministerial Order of 1995, and are based on European Union recommendations. The standard is based on *E. coli*, which must not exceed 2 per 100 ml in 90% of the samples, and 7 per 100 ml in the remaining 10%.

6.4.2 Algeria

Algeria's coast hosts approximately 12.5 million people (1998), representing 45% of the country's population. During the summer months tourists increase the permanent population. Algiers, Oran, Annaba, Ghazaouet, Mostaganem, Arzew, Bejaia and Skikda are the most important coastal cities (NDA Algeria, 2003). Major pollution problems include untreated urban and industrial wastewater, petroleum hydrocarbon slicks and coastal erosion. Most of the urban wastewater is discharged untreated directly into the sea. Although 17 treatment plants for urban wastewater have been constructed in the Algerian coastal zone, only five are in normal operation. This represents approximately 25% of the total treatment capacity. Faecal microorganisms are present on most Algerian bathing beaches, exceeding sanitary standards. Petroleum hydrocarbon pollution is very common along the Algerian coastline because of maritime oil shipping lanes that pass close to the Algerian coast. Erosion is also a major issue. Out of 250–300 km of sandy beaches in Algeria 85% are retreating, losing sand at a rate ranging from 0.30 to 10.4 m/year. There is no information for official shellfish growing areas or official surveillance project running in Algeria to date.

6.4.3 Bosnia and Herzegovina

The Mediterranean coast of Bosnia and Herzegovina on the Adriatic is 25 km long, hosting the town of Neum (population 4300). The pollutants generated in the drainage basins of the major Bosnian rivers of Neretva (from the nearby towns of Konjic, Mostar, Caplinja, Ploce and Metcovic) and Trebisnjica (from the towns of Bileca and Neum) can be carried to the Adriatic Sea affecting its environment (NDA Bosnia and Herzegovina, 2003). The major pollution problems are untreated urban wastewater and occasional stockpiles of obsolete chemicals. The areas of concern are:

- Mostar (population 130000). Urban and industrial wastewater is discharged into the River Neretva without any treatment and urban solid wastes are dumped without proper management. Barrels of obsolete chemicals are left on both riverbanks. During the war (1992–1995), bombing destroyed electric power transformers leading to oil leakage and contamination of soil and water with PCBs.
- Neum (population 4300) is the only urban centre in Bosnia and Herzegovina that discharges its primarily treated urban wastewater directly into the Adriatic Sea. The town population doubles during summer months because of tourism.

Standards for shellfish growing waters in Bosnia are based on European Union standards. There is no valid surveillance programme in Bosnia to date.

6.4.4 Croatia

Croatia has a permanent coastal population of 1,000,000 which increases considerably during the summer because of tourism. The larger coastal towns are Split (population 207000), Rijeka (population 206000), Zadar (population 137000), Pula (population 85000), Sibenik (population 85000) and Dubrovnik (population 71400). Ongoing physical alterations in many areas are the results of intense uncontrolled construction along the coastline (recreational buildings, tourist facilities, marinas and small harbours). This has led to dumping and depositing of inert materials. Another threat to the coastline is fish farming, which has caused habitat degradation in the vicinity of the fish cages and conflicts with the tourist business.

Shellfish water is classified as class 1 in the Water Classification Decree of 1981, where four classes of coastal sea water are established. There are both microbiological and physicochemical parameters. With regard to the former, the acceptable concentration of total coliforms (MPN) per 100 ml should not exceed 100 cfu/ml. Table 17 provides data submitted for the present assessment.

Table 17
Information on Croatia shellfish growing areas

Year	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No. of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
1996	ND	ND	ND	ND	ND
1997	ND	ND	ND	ND	ND
1998	ND	ND	ND	ND	ND
1999	4	3	1		
2000	4	3	1		
2001	4	3	1		
2002	4	3	1		
2003	4	2	2		
2004	4	1	3		
2005	4	1	3		

National legislation:
Based on the EU Directive of 1976 ☒
Based on national standards ☒
Stricter than WHO/UNEP ☒

6.4.5 Cyprus

The southern coastal zone of Cyprus is densely populated by about 370000 permanent inhabitants (47% of the total permanent population) and tourists (3 million per year). The country's industrial sector is small and therefore industrial pollution is limited. All coastal towns and tourist centres operate wastewater treatment plants. The major environmental problems are coastline alteration, industrial mining activities and urban wastewater, in the Bay of Limassol, the Bay of Liopetri and Ayia Napa, and the Bay of Vassilikos (NDA Cyprus, 2003).

There are no standards established by law in Cyprus for shellfish water quality, as there are no officially designated shellfish growing areas in Cyprus. The shellfish used originate from unofficial shellfish growing areas and are consumed locally.

6.4.6 Egypt

The coastal area around Alexandria (Lake Manzala, Abu-Qir Bay and Mex Bay, Alexandrian coast) is the major area of concern in Egypt as is Port Said. Major environmental problems are caused by untreated urban and industrial wastewater and intense urbanization has caused coastline degradation (NDA Egypt, 2003). There are no specific statutory standards or criteria under Egyptian law regarding the microbiological quality of shellfish waters or shellfish. They are however examined at regular annual intervals and their quality evaluated according to international (global) and European standards. Enforcement is through internal administrative procedures from correspondent Ministry of Agriculture. There is no official surveillance project and the most of the shellfish are consumed locally.

6.4.7 France

France's coastline in the Mediterranean extends for 1960 km in the regions of Languedoc-Roussillon, Provence-Alpes-Côte d'Azur and Corsica. Major environmental problems are caused by river transported pollution, and treated industrial and urban wastewater. In addition, intense urbanization along the densely populated coastline is also a major cause for concern (IFEN, 1999). Urbanization of the coastline owing to construction of marinas alters important parts of the natural coastline. Between the towns of Martigues and Menton, 15% of the coastal zone with depths 0 to 10 m and 17% of the coastline (110 km) consists of concrete. Similarly, 20% of the 120 km long coast in the Alpes-Maritimes area is occupied by small harbours, marinas and boat shelters.

French law is based on the new Water Framework Directive (2000/60/EC) and the new European Food Standard Regulations 854/2004. In addition, an internal administrative standard (which has no statutory standing) classifies shellfish waters into four categories as follows:

A:	Satisfactory	0 *Escherichia coli* per 100 ml seawater
B:	Acceptable	1-60 *Escherichia coli* per 100 ml seawater
C:	Doubtful	61-120 *Escherichia coli* per 100 ml seawater
D:	Unsatisfactory	above 120 *Escherichia coli* per 100 ml seawater

Shellfish for consumption are subject to conformity with the following criteria, and there are detailed procedures for interpretation of results. Microbiological standards: Aerobic micro-organisms, 30EC 100,000 per gram; faecal coliforms 300 per 100 ml; faecal streptococci 2,500 per 100 ml; *Staphylococcus aureus* 100 per gram; anaerobic sulphur-reducing bacteria, 46EC 10 per gram; *Salmonella* absent in 25 grams.

For each shellfish group, there is a ranking of shellfish growing zones according to their sanitary quality. The microbiological requirements are based on the provisions of EC Directive 854/2004:

Class A < 300 faecal coliforms *or* 230 *Escherichia coli* per 100 g flesh
Class B < 6000 faecal coliforms *or* 4600 *Escherichia coli* per 100 g flesh
Class C < 60000 faecal coliforms *or* 46000 *Escherichia coli* per 100 g flesh

6.4.8 Greece

The coastline of Greece has a length of approximately 15000 km. It hosts 50% of the country's population and the majority of the industrial activity (NDA Greece, 2003). Most coastal cities operate wastewater treatment plants. Localized environmental problems are caused by poorly treated urban and industrial wastewater, and run-off from agricultural areas. The major source of nitrogen to the marine coastal areas of Greece is run-off from agricultural land, which contributes between 45% (in the Aegean Sea islands) and 70% (in the eastern Peloponnesus) of the total load. The endangered marine coastal areas in Greece are as follows: Greek law originally classified shellfish-growing waters into three categories, (suitable, moderately infected and unsatisfactory) based on total coliform concentrations (up to 70, 71 to 700, and above 700 respectively) in 100 ml seawater. Shellfish for consumption were classified into three categories (first, second and third class) based on concentrations of *Escherichia coli* (up to 500, 500 to 1500 and above 1500 respectively) per 100 ml of flesh. Under legislation enacted in 2004, the quality of shellfish waters in Greece is based on the 1979 EC Directive 854/2004. Apart from the guide standard of 300 per 100 ml flesh and intervalvular fluid for faecal coliforms, Greek law also sets a mandatory standard of 700 per 100 ml. Shellfish satisfying the guide value are acceptable for human consumption, those satisfying the mandatory value are subjected to depuration.

The microbiological quality of shellfish harvested from designated shellfish waters is determined by a Ministerial regulation issued in December 1994 in terms of the provisions of EC Directive 91/492/EEC of 15 July 1991. The zones and parameters are the same as those described for France. Shellfish harvested from Class A zones are considered suitable for consumption, those harvested from Class B and C zones require depuration before consumption. At present, there are only Class A designated zones in Greece. No depuration procedures have been developed for the establishment of Class B and Class C zones. So, all the shellfish that are harvested to other than Class A zones are sent for depuration mainly to Italy. The microbiological quality of shellfish produced in small quantities for the local market (up to 100 kg per day) is determined by health regulations which stipulate that shellfish sampled in the market should not exceed 5 faecal coliforms per ml of flesh to be considered suitable for consumption. Shellfish containing between 6 and 16 faecal coliforms per ml of flesh require depuration before consumption, while those containing more than 16 per ml are considered unsuitable for consumption. The shellfish productions zones in Greece, officially monitored today are 24, mainly located in North Greece. Table 18 shows detailed information.

Table 181
Information on shellfish growing areas in Greece

Year	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No. of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
1996	NA	NA	NA	NA	NA
1997	NA	NA	NA	NA	NA
1998	NA	NA	NA	NA	NA
1999	16	16	0	30	0
2000	18	18	0	24	0
2001	18	18	0	22	0
2002	20	20	0	25	0
2003	21	21	0	29	0
2004	24	24	0	42	0
2005	24	24	0	32	0
2006	24	24	0	45	0

ND: Not applied

National legislation: Based on the EU Directive of 854/2004 ☒
Based on national standards ☒

6.4.9 Israel

Seventy percent of the population resides within 15 km of the Mediterranean coastline, where the major economic and commercial activities are concentrated. The main pollution sources include industrial and urban wastewater, although most of the urban wastewater is treated and recycled. The rivers Na'aman (near the city of Akko), Yarkon and Taninim are transporting nutrients from agricultural run-off. There are no standards for shellfish growing, as shellfish are not grown or harvested in Israel.

6.4.10 Italy

Italy's coastline stretches 7500 km and the whole territory is located in drainage basins flowing into the Mediterranean Sea. Major environmental problems are caused by urban and industrial wastewater, agricultural run-off and shipping. Urbanization of the coastline is also occurring because of tourist infrastructure development. Most cities have wastewater treatment plants, however only 63% of the population is connected to them. Furthermore, 13% of the existing plants have operational problems or need upgrading (OECD, 2002). The river Po is a very important pollution vector in the area transporting urban and industrial wastewater as well as agricultural run-off from its drainage basin to the Adriatic Sea. In the mid 1990s, the nitrogen load transported through the river amounted to 270000 tons per year, leading to eutrophic algal blooms in the area.

Italy follows the European guidelines (854/2004) for shellfish. Shellfish growing waters are still classified into approved zones and conditioned zones, with the following standards:

Approved zones: Sea water should not contain more than 2 *Escherichia coli* per 100 ml. Up to 7 per 100 ml seawater is tolerated in not more than 10% of the samples, provided that the shellfish themselves come up to the required standards. Shellfish should not contain more than 4 *E. coli* per ml of flesh plus intervalvular fluid, and *Salmonella* must be absent in 25 ml flesh plus intervalvular fluid.

Conditioned zones: Seawater should not contain more than 34 *E. coli* per 100ml. Up to 49 per 100 ml are tolerated in not more than 10% of the samples. Shellfish should not contain more then 39 *E. coli* per ml of flesh plus intervalvular fluid.

Depurable species are only cleared for direct consumption if they originate from culture areas in an approved zone. Depurable species originating from (a) natural breeding grounds in approved zones and (b) culture areas in conditioned zones are subject to mandatory depuration prior to consumption. Those originating from natural breeding grounds in conditioned zones must be cooked prior to consumption. Non-depurable species are cleared for direct consumption if they originate from approved zones, or from culture areas in conditioned zones, otherwise they are subject to mandatory cooking. The zones and parameters are the same as for France and Greece, but under Italian Law, Class A zones also have a requirement for *Salmonella* and *Vibrio* spp. (0 in 25 g flesh plus intravalvular fluid). Stabilization zones are also included, with the same standards as for Class A zones. Italy also possesses standards for algal biotoxins in shellfish. For DSP, waters must contain less than 1000 *Dinophysis* per litre, and shellfish must conform to a death time of more than five hours. The limit value for PSP is 40 µg per 100 g flesh. The official controls for biotoxins and also microbiological quality are performed by several different laboratories controlling separate regions such as: Regione Emilia-Romagna, Regione Veneto, Regione Friuli Venezia Giulia as well as Istituto Zooprofillatico. Table 19 gives detailed information on shellfish quality in Italy.

Table 19
Information on shellfish quality in Italy

	Total no. of samples	**Samples NC[1]**	**Samples C[2]**
1999	630	102	528
2000	700	79	621
2001	842	117	725
2002	736	242	494
2003	766	126	640
2004	673	200	473
2005	731	26	705
2006	616	49	567

[1]NC = non-complying with the national and European legislation
[2]C = complying with the national or European legislation

6.4.11 Lebanon

It is estimated that 2.3 million people are resident in the Lebanese coastal zone. This zone is very narrow and lies between the west mountainous chain and the sea. Major pollution problems are untreated urban wastewater, solid wastes and coastline urbanization. Beirut, Tripoli, Sidon, Jounieh and Tyre are the major coastal cities. Urban wastewater is discharged into the sea untreated (44000 tonnes of BOD5 per year) as no municipal WWTP is in operation in the country (NDA Lebanon, 2003). The major factor for the physical alteration of the coastal zone is urbanization since most of the coastal fringe (at a width of 8 to 10 km) is built-up. There is no information for shellfish production areas.

6.4.12 Libya

Libya's coastal zone hosts 85% of the country's population and most of its industrial, agricultural and tourist activity (NDA Libya, 2003). There are no natural rivers in the area, only wadis (temporary dry rivers) which transport sediment, litter and pollutants from inland to the sea during storms. With the exception of the larger coastal cities, most towns have no effective sewer system. Therefore, discharge of wastewater into the sea is minimized. Major environmental problems in Libya are oil pollution near terminal facilities as well as untreated urban and industrial wastewater from the bigger cities. There are no national standards for shellfish waters in force in Libya. Pending the development and adoption of new standards, which are currently being finalized, Libya is observing the standards adopted by the Contracting Parties in 1987. Table 20 provides data submitted for the present assessment.

Table 20
Information on shellfish growing areas in Libya

Year	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No. of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
2005	Abu Kammash - Zwara - Sabrata	Abu Kammash - Zwara - Sabrata		Shellfish, will not be consumed	

National legislation: Based on WHO/UNEP interim quality criteria of 1985 ☒

6.4.13 Malta

Malta has a coastline of 190 km, 43% of which is heavily utilized (the remaining 57% being inaccessible). The built-up area comprises 24% of the coast. This constitutes a very high population density (1300 persons/km^2). The southern part of the island of Malta is the area with the majority of human activities (cities, harbours, and tourist resorts) and the major environmental problems, i.e. urban and industrial wastewater. On the island, 85% of urban and industrial wastewater is disposed of untreated while solid wastes are mainly disposed of in two landfill sites (Malta Ministry of Environment, 2001; Malta National Statistics Office, 2002).

There are no standards fixed by law in Malta for shellfish water quality. The quality of shellfish for consumption comes under general Public Health legislation, and the sale of shellfish consignments is prohibited unless the person concerned is in possession of a special permit from the Superintendent of Public Health. There are currently no valid permits for the sale of fresh (as distinct from imported) shellfish.

6.4.14 Monaco

Monaco has a population of 33000, and a high population density (16500 people per km^2). The city wastewater (urban and industrial) is discharged into the sea through submarine outfalls after treatment. Furthermore, there is also primary treatment of storm water before it is discharged into the marine environment. Solid wastes are recycled (glass, paper, batteries, lubricating oil) or incinerated, reducing their weight by 70% before sanitary disposal. Special industrial wastes are also treated (Principauté de Monaco, 1997). The greater part of the coastline of Monaco is urbanized. There are no official shellfish growing areas.

6.4.15 Montenegro

The Mediterranean coast of Montenegro has a population of 409000. Four percent of the total population of the country resides in urban areas. The major towns are: Bar (population 47000), Herceg Novi (37000), Kotor (23000), Ulcinj (21500), Budva (18000) and Tivat (15600) (NDA Serbia and Montenegro, 2004). The summer population of these towns increases because of tourism. Owing to the discharge of untreated urban wastewater, eutrophication problems and microbial pollution can be detected in the vicinity of coastal towns (west beaches of Bar, Herceg-Novi Bay, Kotor Bay, Port Milena [Ulcinj] and Tivat Bay). Similar problems exist at Velika Plaza and Ada at the river mouths.

6.4.16 Morocco

The Mediterranean coast of Morocco has witnessed increased urbanization over recent years. From 1977 to 1994, medium-sized coastal towns grew from 16 to 30, and small towns from 2 to 14. The major urban centers, which are also the most polluted areas on the Mediterranean coast, are: Tangiers (population 640000), Tetouan (333000), Nador (149000) and Al Hoceima (65000) (NDA Morocco, 2003). The main environmental problems are caused by urban and industrial wastewater, maritime traffic and coastal urbanization. For example, construction, sand extraction and erosion have resulted in serious stress on the beaches. This has led to the disappearance of seven out of 47 beaches in recent years. The major beaches under stress are in Tetouan, Mdiq, Restinga-Smir, Al Hoceima, Cala Iris, Nador and Essaidia. Maritime traffic is one of the major concerns for oil and hazardous compounds contamination. It is estimated that 60000 ships pass through the straits of Gibraltar yearly, including 2000 ships carrying chemicals, 5000 oil tankers and 12000 gas tankers.

Microbiological quality standards and criteria for shellfish waters are generally based on French legislation. For acceptability of shellfish waters, the concentration of faecal coliforms in the flesh of shellfish therein must not exceed 300 per 100 ml, in line with the 1979 EC Directive and the 1987 Mediterranean standards. Table 21 provides data submitted forth present assessment.

Table 21
Information on shellfish waters in Morocco

Year	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No. of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
2001	Axe Fnidek – Kaâ Srass Axe cap de l'eau – Saidia	Fnidek –M'diq, M'diq– Kaâ Srass cap de l'eau – Saidia	Martil Area of the lagoon of Chaala		
2002	Axe oued laou – Kaa srass Axe Kabila – M'diq	oued laou – Kaa srass Kabila – M'diq	Martil		
2003	Axe oued laou – Kaa srass Axe Kabila – M'diq	oued laou – Kaa srass Kabila – M'diq	Martil		
2004	Axe oued laou – Kaa srass Axe Kabila – M'diq	oued laou – Kaa srass Kabila – M'diq	Martil	June-December (PSP in the shellfish May-June (PSP in the shellfish)	
2005	Axe oued laou – Kaa srass Axe Kabila – M'diq	oued laou – Kaa srass Kabila – M'diq	Martil	January-Mars (PSP in the shellfish)	

National legislation: Based on the EU Directive of 1979 ☒
Based on national standards ☒

6.4.17 Slovenia

Slovenia possesses a short coastline on the Adriatic Sea (46.6 km). It hosts approximately 80000 people who mainly reside in the towns of Koper, Izola and Piran. More than 80% of the Slovenian coastline is urbanized and mostly within 1.5 km from the sea front. This leaves only 8 km (18%) of coast in its natural state. Major environmental problems are related to discharge of partly treated urban and industrial wastewater and run-off from agricultural land (NDA Slovenia, 2003). Water quality control instructions for shellfish breeding are contained in the 1988 Slovenian Decree of 1988 on Preventive Vaccination, Diagnostics and Research in the Relevant Field. The standard for acceptable shellfish waters is 10 faecal coliforms per 100 ml flesh, based on a fortnightly sampling frequency. Table 22 provides data submitted for the present assessment.

Table 22
Information on shellfish quality

	No. of shellfish growing areas monitored	Total no. of samples	Samples NC[1]	Samples C[2]
1996	6	42	13	29
1997	6	30	8	22
1998	5	31	6	25
1999	6	29	8	21
2000	2	3	1	2

[1]NC = non-complying with the national legislation*
[2]C = complying with the national legislation*
* Decree on the classification of the water of inter-republic watercourses, international water and coastal sea water of Yugoslavia (Official Gazette of the Socialist Federal Republic of Yugoslavia, no. 6/1978), which stipulated that shellfish growing waters may contain 100 (MPN) coliforms per litre of water at the most (provisions not in force since 11.06.2002)

6.4.18 Spain

The Spanish Mediterranean coast has a population of 15.6 million, representing more than 39% of the country's population. Urbanization is very intense as 85% of the Mediterranean coastal population lives in cities and towns of more than 10000 people. The major cities are: Barcelona (4 million), Valencia (2.1 million), Malaga (900000), Murcia (400000), Palma de Mallorca (370000), Granada (310000), Cartagena (185000), Benidorm (125000), Tarragona (110000) and Algeciras (105000) (UNEP/Plan Bleu, 2001).

Under Spanish law, the limits regarding the acceptability of shellfish waters from the point of view of their microbiological quality were that concentrations of *E. coli* should not exceed 15 per 100 ml of seawater in more than 50% of the samples and 50 per 100 ml of seawater in more than 10% of the samples. By Royal Decree 38/1989, Spain set values for the parameters listed in the annex to the 1979 EC Directive. The standards are the same as those in the Directive. Shellfish areas are now also classified into three zones according to the terms of the 1991 EC Directive, the parameters and limit values being the same as described for France and Greece. Depurated shellfish destined for consumption must comply with the following microbiological standards:

Aerobic micro-organisms:	up to 100,000 per gram
Escherichia coli	up to 500 per litre
Salmonella	absent in 25 ml
Streptococci (Group D)	up to 100 per gram
Vibrio parahaemolyticus	up to 100 per gram

6.4.19 Syria

The Syrian coastal area represents only 2% of the country's surface but hosts 11% of its population (i.e. 1.5 million). The major coastal cities are Lattakia, Jableh, Tartous and Banias. Coastal urbanization, due to housing needs (local and tourist) and industrial development, (harbour facilities) has led to serious environmental problems. These problems are: disposal of untreated urban and industrial wastewater, oil slicks from the oil refinery and the oil terminal, and the management of solid wastes (NDA Syria, 2003). In total it is estimated that 24.8 million m^3 of urban wastewater, 99% of which is untreated, is discharged into the sea.

6.4.20 Tunisia

The coastal zone of Tunisia is densely populated, hosting 6.3 million (70.2% of the country's population) in 1995. Tunis (population 1.6 million), Sfax (510000), Sousse (185000), Gabes (140000) and Bizerta (130000), are the most important cities. A major part (81%) of the cities' wastewater is treated. The major environmental problems are industrial and urban wastewater, industrial (phosphogypsum) and urban solid wastes, and coastal urbanization.

Shellfish-growing waters are classified into three categories:

Sanitary zones: Shellfish flesh up to 300 faecal coliforms per 100 ml, Salmonella absent in 25 g, Water, up to 2 faecal coliforms per 100 ml.

Conditioned zones: Shellfish flesh up to 3900 faecal coliforms per 100 ml, Water, up to 34 faecal coliforms per 100 m

Unsanitary zones: Shellfish flesh above 3900 faecal coliforms per 100 ml, Water, above 34 faecal coliforms per 100 ml.

The information related to shellfish water quality is included in Table 23. Regularly, 16 shellfish growing sites were monitored. For 1996 and 1997, there was no classification for growing shellfish water, while from 1998 until 2002 all the sites showed 100% compliance with the regulation. During 2003 and 2004, three sites did not comply with the regulations according to Tunisian laws, where as in 2005 only two sites did not comply. All the sites that were found inappropriate were closed, and they were opened again when the situation in the water quality improved and met the normal conditions.

Table 23
Shellfish growing waters : Collection Areas of Palourde (*Tapes decussatus*)

Years	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No. of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
1996	16 areas	Not classified	-	-	-
1997	16 areas	Not classified	-	-	-
1998	16 areas	16 areas	0	0	0
1999	16 areas	16 areas	0	0	0
2000	16 areas	16 areas	0	0	0
2001	16 areas	16 areas	0	0	0
2002	16 areas	16 areas	0	0	0

2003	19 areas	16 areas	3	0	0
2004	19 areas	16 areas	3	0	0
2005	19 areas	17 areas	2	0	0

Based on national standards ☒
Decree of the Ministry of Agriculture of 28/11/1995 setting sanitary regulations managing the production and the trade of alive bivalve molluscs

6.4.21 Turkey

The Turkish coast extends for 8.333 km and can be divided into the Aegean region and the eastern Mediterranean region. Urban and industrial centres, oil terminals, agricultural and recreational facilities on the coast are the major land-based pollution sources in both regions (NDA Turkey, 2003). Rapid urbanization is taking place in Turkey because of recreational constructions and extensive building of second (vacation) houses on the Aegean and eastern Mediterranean coastline. This is drastically altering the landscape. Coastal erosion is also an important problem. Out of 110 sand dune systems recorded in the 1980s only 30 (27%) are relatively intact today. Areas of concern include: The Aquatic Products Law, which came into force in 1971, contains general conditions and regulations for coastal protection and production of aquatic products. The Aquatic Products regulations entered into force in March 1995. This law regulates, *inter alia*, the discharges to fish and shellfish production areas and tolerable values in receiving waters. Table 24 provides data submitted for the present assessment. The Ministry of Health is responsible for the coordination of activities related to aquatic products at both national and international levels.

The Quality Control System for fishery products was formed in 1998 and has been developed under the Fishery Law, the Fishery products regulations and European Union Directives (91/493/EEC, 91/492/EEC, 79/223/EEC and 94/356/EEC) and the FAO Standard (Codex Alimentarius). There are two classes and four regions in Turkey (1999/767/EC Decision); Two in A class (live bivalves and molluscs-91/492/EEC) and two in B class. The current procedure for opening and closing areas as well as routine monitoring is discussed in terms of the fishing season that generally lasts from 01 September to 01 May. The difficulties in applying a monitoring programme include the lack of a clear limit for some parameters, sampling in bad weather conditions. The annex to the regulations defines limits on activities and substances. The microbiological limits for receiving waters are:

Total coliforms	not to exceed 70 per 100 ml
Faecal coliforms	not to exceed 10 per 100 ml
Escherichia coli	not to exceed 2 per 100 ml (extendable to 7 per 100 ml).

Table 24
Information on shellfish waters in Turkey

Year	Shellfish growing areas regularly monitored	Shellfish growing areas complying with standards	Shellfish growing areas not complying with standards	No of prohibited days for trade of shellfish due to high contamination of the shellfish itself	Epidemics due to consumption of contaminated shellfish
1996	-	-	-	-	-
1997	-	-	-	-	-
1998	30	30	-	At 15 shellfish growing areas out of 30 of 944 days in total	-
1999	26	26	-	At 12 shellfish growing areas out of 26 of 1212 days in total	-
2000	32	32	-	At 15 shellfish growing areas out of 32 of 1206 days in total	-
2001	28	28	-	At 14 shellfish growing areas out of 28 of 547 days in total	-
2002	37	37	-	At 20 shellfish growing areas out of 37 of 1280 days in total	-
2003	36	36	-	At 27 shellfish growing areas out of 36 of 1500 days in total	-
2004	43	43	-	At 34 shellfish growing areas out of 43 of 2635 days in total	-
2005	37	37	-	At 20 shellfish growing areas out of 37 of 1042 days in total	-

National legislation: 1. Based on the EU Directive of 1979 ☒

7. HEALTH IMPLICATIONS OF CONTAMINATED SHELLFISH WATERS

Human activities threaten the ecosystems of the Mediterranean Sea. Only a small area of its coastal zone is in pristine condition. Contamination originates from eutrophication, heavy metals, organic and microbial pollution, oil spills and introduction of non-indigenous species. While efforts have led to positive developments both at national and regional level, the overall status of the Mediterranean environment still shows the need for specific and immediate action. Water-based recreation can expose individuals to these contaminants as well as a variety of other health hazards, including physical hazards and effects of toxic products from algae and other organisms.

The Mediterranean is particularly prone to contamination due to its popularity as a tourist destination. The climate in the Mediterranean is mild year-round and therefore the bathing season is extended in comparison to northern European destinations. Nevertheless in Mediterranean destinations, the weather conditions and the holiday calendar in European countries tend to create a strong seasonal pattern resulting in pressures on the environment at certain periods of the year.

It is estimated globally that 2 billion man days are spent at coastal recreational water resorts (Shuval, 2003). The World Tourism Organization predicts that by 2026, 346 million tourists will visit Mediterranean destinations annually, representing 22% of all arrivals worldwide (WTO, 2001).

Shuval (2003) estimated that globally, each year, there are in excess of 20 million cases of gastrointestinal disease and in excess of 50 million cases of more severe respiratory diseases caused by swimming and bathing in wastewater-polluted coastal waters. Shuval (2003) also estimates that annually there are around 4 million cases of infectious hepatitis A and E with around 40,000 deaths and 40,000 cases of long-term disability, mainly chronic liver damage, from consuming raw or lightly steamed filter feeding shellfish/molluscs harvested from polluted coastal waters globally. A rough estimate of the total global health impact of the human infectious diseases associated with pathogenic micro-organisms from land-based wastewater pollution of the seas – is about 3 million disability-adjusted life years (DALYs)/year, with an estimated economic loss of around12 billion dollars per year (Shuval, 2003).

7.1 Bacteria

The earliest reports of shellfish-transmitted bacterial diseases were documented in the late 19th and early 20th century. Numerous outbreaks of typhoid fever in Europe, the United States, and elsewhere have been linked to sewage pollution. Other bacterial agents have also been found to cause disease after raw shellfish consumption. The most important examples include *Vibrio* species, which account for 20% of all outbreaks of disease. Other bacteria are *Salmonella* species, *Shigella* species, *Plesiomonas shigelloides,* and *Listeria* species.

Vibrio species are halophilic, non–spore-forming bacteria that grow in saline aquatic environments. They produce a wide range of clinical symptoms. Specifically, *Vibrio vulnificus* infections can result in wound infection and septicemia with a high mortality rate. Other species are associated with gastroenteritis of varying severity, although it is usually much more severe than gastroenteritis associated with other viral pathogens. Among *Vibrio* species, infection with *Vibrio cholerae* O1 is the most serious and debilitating. *V. vulnificus* has been associated with both ingestion of contaminated seafood, such as raw oysters and clams, or infectious wounds contaminated by seawater. Oyster-associated *V. vulnificus* sepsis and death were first reported in 1979. Both outbreaks and sporadic cases of infection have been reported in the United States. During 1965–1991, 136 cases of infection due to *V. cholerae* O1 were reported in association with shellfish ingestion. *Vibrio parahaemolyticus* infection is quite commonly associated with eating undercooked shellfish. It is the leading cause of *Vibrio*-associated gastroenteritis in the United States. *V. parahaemolyticus* gastroenteritis infections are frequently reported, but most cases are probably sporadic. DePaola *et al.* (1990) investigated the occurrence of *Vibrio parahaemolyticus* in shellfish growing waters in United States. They found no correlation of *V. parahaemolyticus* with faecal coliforms. Concentrations were 100 times greater in oysters than in the water. Temperature appeared to be a significant factor in the seasonal and geographical distribution of this organism.

Campylobacter, Salmonella, and *Shigella* species and *E. coli,* which are commonly implicated in gastroenteritis, are only occasionally traced to seafood. One outbreak and several sporadic cases of infection have been reported to be due to *Plesiomonas shigelloides. P. shigelloides* is interesting in several aspects. First, it is mostly an organism of fresh water and estuaries. Second, the clinical picture of infection may vary from mild diarrhoea to bloody diarrhoea with faecal leukocytes and, occasionally, extra intestinal manifestations. Third, the laboratory identification from mixed cultures requires selective media.

With a better appreciation of the limitations of the use of indicators, new methods are being used to detect the presence of bacterial pathogens in shellfish growing waters. In a study in Spain, *Salmonella* was detected in 32 percent of 256 samples collected from 21 bathing beaches along the north east coast (Perales and Audicana, 1989). Similarly, 16 sites in New York Harbour were sampled for the presence of *Salmonella* (Knight *et al.*, 1990). Salmonellae were detected at 75 percent of the sites and in 50 percent of these samples, cultivation techniques failed to isolate the organism. Previous work has demonstrated that non-cultivatable organisms can remain infectious (Grimes and Colwell, 1986).

Newly recognized bacterial pathogens have also been studied in coastal estuarine waters. *Listeria monocytogenes* has been associated with foodborne gastroenteritis. This organism was detected in 62 percent of the samples in the Humboldt-Arcata Bay in California. The organism was found in 17 percent of the sediment samples but was not detected in oysters. It was suggested that domestic animals, such as horses and cattle, were responsible for the contamination (Colburn *et al.,* 1990).

Salmonella, Yersinia, and *Campylobacter* are associated with animal reservoirs. *Salmonellae* are common in poultry (chickens, turkeys, and ducks) and in gulls, pigeons, and doves but have been identified in other wild birds much less frequently (Feachem *et al.*, 1983). Between 15 and 50 percent of domestic animals and 10 percent of mice and rats may be infected. Wild mammals do not appear to be a major source for human infections. Both wild and domestic animals may serve as reservoirs for *Yersinia enterocolitica.* The organism has been identified in foxes and beavers as well as cattle, sheep, and pigs. *Campylobacter* has been found in a wide variety of animals. Domestic animals (cattle, sheep, and pigs) and birds (poultry and caged birds) have been documented as sources of infections in humans.

Animals may also contribute significant numbers of indicator bacteria (total coliforms, faecal streptococci, and enterococci) to waters (Crane *et al.* 1983). Gannon and Busse (1989) suggested that animals were the source of the elevated indicator bacterial levels in storm water. An epidemiological study of recreational waters has suggested that the indicator bacteria arising from agricultural inputs are not associated with human bacterial and viral infections (Calderon *et al.,* 1991).

7.2 Viruses

Many viruses transmitted by the faecal–oral route are widely prevalent in the community and infected individuals can shed millions of virus particles in their faeces. Consequently viruses, of many types, occur in large numbers in sewage. Sewage treatment processes, if present, are only partially effective at virus removal (Sorber, 1983), therefore coastal discharges constantly release human viruses into marine environment.

Following shedding into the environment viruses can survive for weeks to months (Gantzer *et al.,* 1998) either in the water column or by attaching to particulate matter and accumulating in sediments. Thus seafood species harvested from sewage polluted areas may potentially be contaminated with human enteric viruses. However, a number of factors determine whether such potential contamination constitutes a health risk. Major factors include whether viral contamination remains on the surface or becomes internalised, and if so whether such contaminated organs are consumed or are removed during handled. The role of shellfish as vectors in human enteric virus diseases is well-documented. Epidemiological evidence gathered in recent years suggests that human enteric viruses are the most common pathogens transmitted by bivalve shellfish. Despite the multiplicity of viruses transmitted faecal-orally, only a handful of viruses have been linked to diseases caused by bivalve consumption.

Viruses which have been shown epidemiologically to be transmitted by shellfish are Hepatitis A and E (the latter of which, however, is not endemic to the Mediterranean region), Noroviruses, Astroviruses, Coxsackie viruses and small round viruses. Of these, Hepatitis A and Noroviruses appear to be of chief concern to public health officials. There are a number of reports worldwide of gastrointestinal disease due to eating shellfish for which no causative agent has been identified, and many of these cases were believed to involve an unidentifiable viral agent, rather than a bacterial pathogen. Well-known examples of agents are NLVs and small round structured viruses (SRSVs), such as astrovirus and coxsackievirus, and hepatitis A virus (HAV), hepatitis E virus (HEV), and poliovirus. Furthermore, once these viruses are inside the shellfish, their survival appears to be further prolonged for weeks, and they can withstand depuration.

NLVs and SRSVs belong to genus 1 of the caliciviruses. This group of small viruses (diameter, 30–35 nm) contains a single stranded RNA. The caliciviruses include the prototype of Norwalk virus, and other strains, such as the Southampton virus, SRSV, and less well-characterized strains. These viruses appear to have a global distribution. They cannot be grown on culture by means of conventional methods, but they can be identified by electron microscopy and by such recent molecular techniques as RT-PCR, which has led to a better understanding of the epidemiological importance of these pathogens. Caliciviruses emerged as the predominant cause of gastroenteritis associated with shellfish consumption. HAV infection is the most serious viral infection linked to shellfish consumption, causing a debilitating disease and, occasionally, death. The first documented outbreak of "infectious hepatitis" occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption. Subsequently, many HAV-associated outbreaks have been reported worldwide.

The most demonstrative one was the outbreak of HAV infection in Shanghai, China, in 1988, in which almost 300,000 cases were linked to the consumption of clams harvested from a sewage-polluted area. In fact, this is the largest virus-associated outbreak of food poisoning ever reported. Smaller outbreaks have been reported from the United States, Italy, and Australia. The fairly protracted incubation period (mean duration, 4 weeks) of HAV infection makes it very difficult to determine the association with a particular food vehicle in sporadic cases. Cases of infection due to non-A, non-B hepatitis viruses have also been linked to shellfish consumption. HEV shares morphological and biophysical characteristics with caliciviruses.

A few reports link astrovirus infection to shellfish consumption. However, the role of these pathogens seems comparatively minor because of the high levels of adult immunity.

Sapporo viruses are genetically distinct from the faecal–oral route have been associated with shellfish NLVs (Liu *et al.*, 1995) and, although studies are at an early stage, may comprise several distinct strains or from epidemiological evidence linking them with (Jiang *et al.*, 1997). Sero-epidemiological studies have shown a world-wide distribution for these viruses with a high seropositivity rate in adults (Nakata *et al.*, 1996). Sapporo viruses cause sporadic individual cases and occasional outbreaks of diarrhoea illness. Human enteric caliciviruses are a group of related mainly in infants and young children less than 5 years old, and also in the elderly.

NLV (Kapikian, 1994) comprises a genetically diverse group of virus strains separated into genogroup I, containing the prototype Norwalk virus and genogroup II, containing Snow Mountain and other strains (Ando *et al.*, 1994). Over recent years NLV genogroup II frequently have tended to be more prevalent (Fankhauser *et al.*, 1998; Maguire *et al.*, 1999).

Until, recently NLV had only been recognised as human viruses. However, similar diarrhoea causing viruses, closely related by morphological and molecular criteria have now been recognised in cattle (Dastjerdi *et al.,* 1999; Liu *et al.,* 1999) and also possibly in pigs (Sugieda *et al.,* 1998). Human NLV is now recognised to be a major cause of epidemic gastrointestinal illness occurring in families or in community wide outbreaks. Infections occur in all age groups. The evidence clearly suggests that NLV is the most common cause of non-bacterial gastroenteritis in adults (Vinje and Koopmans, 1996). Furthermore recent data from the UK suggests that NLV is the most significant cause of outbreaks of the intestinal disease, causing 43% of outbreaks during 1995 and 1996 compared with 15% for salmonellas (Evans *et al.,* 1998), and also appears to be the most important cause of infectious intestinal disease in the community (Tompkins *et al.,* 1999). As far as is known these viruses are prevalent throughout the world. Unlike common causes of viral gastroenteritis in children, such as rotavirus, atrocious and enteric adenovirus, NLV also frequently causes symptomatic infection in adults. NLV has been linked with diarrhoea and vomiting following seafood, and in particular bivalve molluscan shellfish, consumption.

Astroviruses have been implicated in outbreaks where food and water (Oishi *et al.,* 1994) and seafood (oyster) (Yamashita *et al.,* 1991) were vehicles of infection. The importance of astroviruses as causative agents of gastroenteritis following seafood consumption is therefore currently unclear.

The group A rotaviruses therefore normally present as pediatric infections occurring in infants or and young adults. Group A rotaviruses are common and viruses are shed in large numbers in stools leading to readily detectable virus presence in sewage effluents and polluted receiving waters (Dubois *et al.,* 1997). Consequently rotavirus presence has also been demonstrated in bivalve shellfish grown in contaminated waters. However rotaviruses have not been linked with infectious disease following seafood consumption. Age related resistance to severe infection may be due to active immunity reinforced by repeated infection of the family Picornaviridae (Bishop, 1996).

Although adenoviruses can be detected in polluted sewage effluents, in seawater and in shellfish (Vantarakis and Papapetropoulou, 1998; Pina *et al.,* 1998b) no seafood related outbreaks have been reported. This is presumably because enteric adenoviruses are not generally associated with gastroenteric disease in adults and, for the most part, young children are not primary consumers of seafood.

Commonly such acute symptoms occur only in a small proportion of those suffering an enterovirus infection. It should be noted that although enteroviruses replicate in the intestinal tract, and are transmitted by the faecal–oral route, they do not commonly cause classical gastroenteric symptoms such as diarrhoea grown in culture, wild hepatitis A.

The significance of viral contamination of marine waters, and currently the scientific consensus supports several conclusions:

- Enteric viruses persist significantly longer when compared with the bacterial indicators;
- There is no qualitative or statistical association between the enteric viruses and the bacterial indicators, and
- Enteric viruses have been isolated from both waters and shellfish within current bacterial standards for water quality.

7.3 Parasites

There is not much information available on risks to human health arising from the presence of animal parasites in the marine environment. Protozoan parasites of either worldwide distribution or present in the Mediterranean region include *Entamoeba histolytica,* *Giardia lamblia*, *Cryptosporidium parvum*, *Balantidium coli* and *Naegleria* species among those present in sewage and constituting a potential health hazard.

C. parvum, *G. duodenalis* and human-infectious microsporidia (i.e., *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi*) are human enteric pathogens that inflict considerable morbidity on healthy people and can cause mortality in immunosuppressed individuals. The transmissive stages of these pathogens, i.e., oocysts, cysts, and spores, respectively, are resistant to environmental stressors and are therefore long lasting and ubiquitous in an aquatic environment. Their viability in seawater lasts from several months up to 1 year. *Cryptosporidium* and *Giardia* are transmitted via water, which is also involved in the transmission of microsporidian spores.

It has been recommended that particular attention should be devoted to these and to nematode eggs when monitoring shellfish harvested in the vicinity of sewage outfalls WHO/UNEP, 2004). Also the eggs of *Ascaris*, *Toxoplasma*, *Oxyuris* and *Trichurus* are able to survive for months in the marine environment, and ingestion of a single egg is sufficient to cause infection. All four nematode species mentioned above are prevalent in the Mediterranean region. The eggs are discharged in faeces by infected individuals, and transmission by swimming in polluted water is a possibility. Interest in *G. duodenalis* and *C. parvum* has increased considerably in recent years, partly with recognition of their enteropathogenicity, and partly with the publication of many reports of episodes of cryptosporidiosis and giardiasis both among indigenous populations and travellers to many localities. They are now recognised as the most common intestinal parasite in developed countries which affects all ages and all socio-economic classes (Barua, 1990). Transmission of parasites is mainly by contaminated water, as the cysts survive in cold waters for more than three months.

Parasites are poorly transmitted via shellfish. Only a few parasites have been isolated from seafood, predominantly fish. These parasites include *Giardia duodenalis* and *Cryptosporidium* species. Oocysts of *Cryptosporidium* species, chiefly *C. parvum,* are the only ones that have been isolated from oysters to date. *C. parvum,* a zoonotic waterborne protozoan, was found in oysters collected from commercial oyster harvesting sites. The oocysts were identified by both PCR and immunofluorescent antibody microscopy. Their infectivity at 20°C could be retained for a period of up to 3 months. The best way to eliminate shellfish-associated infectious outbreaks infectivity was found to be heating to temperatures of 172°C or freezing to -20°C for 24 h. Although *Cryptosporidium* species caused the largest waterborne outbreak of infection in the history of the United States, no clinical outbreaks due to *Cryptosporidium* species have been linked to shellfish. *Cryptosporidium* infection is probably under diagnosed, as is the case with other clinical entities.

C. parvum is found widely distributed in mammals, and zoonotic (animal to human) transmission has been well documented (Current, 1987). Infections in cattle along with rainfalls, which washed the oocyst (the environmentally resistant and infectious form of the organism) into the water supply, were hypothesized as contributing to a large outbreak in the United Kingdom, resulting in 55,000 illnesses (Smith and Rose, 1990).

7.4 Toxic algae

Marine biotoxins cause a large number of poisonings in humans annually, many with serious implications, causing frequent fatalities. Most of these poisonings are in the subtropical/tropical circum global belt region bounded by Florida, the Mediterranean and Japan in the north and the northern edge of Australia, the southern tip of Africa and Chile in the South. The human diseases most frequently associated with marine biotoxins are amnesic shellfish poisoning (ASP), paralytic shellfish poisoning (PSP), ciguatera poisoning, and the more recently identified neurotoxic shellfish poisoning (NSP) and diarrheic shellfish poisoning (DSP). Most of these diseases are apparently associated with fish and seafood that feed on toxic marine algae and toxic algae blooms such as red tides. PSP in particular can lead to severe neurotoxic effects, paralysis and death. The death rate for PSP and some of the other marine biotoxin diseases appears to be in the range of 10%-20% or higher. Serious long-term sequelae, such as neurotoxic effects and paralysis, are common. There have been numerous local reports of outbreaks, and of high endemic incidence, of ciguatera poisoning in small communities and islands in the Pacific, such as Tahiti, Hawaii, Samoa and New Guinea, where the incidence has been estimated to be about 500 per 100,000 population. The case-fatality rate is low (about 0.1%). It is estimated that the total population in the circum global belt where the disease is endemic is about 400 million people, 10% of whom live near sea-coasts and frequently eat locally caught fish and seafood. In Canada, which has one of the best marine biotoxin monitoring and control programs, there are an estimated 1000 cases per year of illness caused by seafood toxins, with 150 cases per year of PSP and 350 cases of ciguatera poisoning. Blooms of toxic algal species are common occurrences in shellfish-growing areas worldwide, the algal species involved, which produce potent toxins, mainly belonging to the dinoflagellate group. The shellfish accumulate the toxic cells during filter feeding, becoming vectors in various forms of shellfish poisoning (Shumway and Hurst, 1991). Of all shellfish consumed, mussels probably pose the greatest threat with regard to shellfish poisoning diseases (UNEP/FAO/WHO, 1995) include the following:

Paralytic shellfish poisoning (PSP), which is caused by a number of toxic components falling into three into three chemical groups, of which the carbamate toxins - Saxitoxin, neosaxitoxin and gonyautoxin - are the dominant component in shellfish, saxitoxin being the first component identified. They are produced by a well-defined dinoflagellate group, mainly *Gonyaulax* (also known as *Alexandrium*) and *Gymnodinium* species, occurring in both tropical and temperate sea. The toxins usually have little effect on shellfish, but are potent neurotoxins to vertebrates, including man, causing respiratory paralysis and death by asphyxia.

Blooms of dinoflagellates producing PSP group toxins have been reported from various parts of the Mediterranean. 26 cases of algal blooms along the French Mediterranean coast in which *Gonyaulax* and *Gymnodinium* species were present have been described (Belin *et al.,* 1989). *Gymnodinium catenatum* was present along the Andalusian coast of Spain in early 1989 (Bravo *et al.,* 1990), resulting in the presence of toxins in the marine bivalves *Venus verrucosa* and *Cytherea*, This was the first record in Spain's Mediterranean coast. The same species was recorded in Volos, Greece, in July 1987 (Gotsis-Skretas, 1988) and in the lagoon of Fusaro, near Naples in 1988 (Carrada *et al.,* 1988). *Gymnodinium* species have also been recorded from the Northwestern Adriatic practically every year between 1976 and 1985 at maximum concentrations of 230 x 106 cells per litre (Mancini *et al.,*1986), in the Bay of Pula (Degobbis, 1990), and from Lake El-Mellah, in Eastern Algeria (Samson Kachacha and Touahria, 1992),

Gonyaulax polyhedra blooms have been recorded several times between 1977 and 1985 in the Northwestern Adriatic (Mancini *et al.,*1986) and in the Gulf of Trieste since 1977 (Fonda Umani, 1985). Algae responsible for red tides in the Emilia-Romagna region of the Adriatic in 1984 were identified as *Gonyaulax polyhedra* (Fortuna *et al.,* 1985). Records of blooms of the same species in the Eastern Adriatic include Pula Bay (Degobbis, 1990), Sibenik Bay (Legovic *et al.,*1991a, 1991b) and Kastela Bay, near Split, in which in the latter locality, blooms are stated to have occurred for the last 30 years. Records of *Gonyaulax polyhedra* blooms from other Mediterranean areas include the Gulf of Kavala, Greece in August 1986 and regularly between April and June along the Western coast of Turkey, during May and June the blooms also containing *Gonyaulax spinifera* (Koray, 1990; Koray *et al.,*1992), *Gonyaulax polyhedra* has also been recorded along the coast of Lebanon although concentrations are low and no health problems have been reported (Lakkis, 1991). Blooms containing *Gonyaulax* species have been recorded from Lake El-Mellah, in Eastern Algeria

(Samson Kachacha and Touahria, 1992). PSP toxins caused by *Gonyaulax tamarensis* in mussels have also been reported from Spain (Shumway, 1990). From a total of 128 samples of seawater in shellfish culture areas in Greece, *Gonyaulax* and *Gymnodinium* species were only found in 12 and 18 samples respectively, the former in low and the latter in relatively high numbers. Concentrations of Saxitoxin both in these samples and in shellfish collected from the market were below detection limits (Papadakis, 1991). *Alexandrium minutum* in mussels is reported as causing the first recorded case of PSP in France in 1989 (Shumway, 1990). The same species has been observed as a large bloom (28 x 106 cells per litre) in the harbour of San Carlos de la Rapita, south of the Ebro delta in Spain in May 1989, PSP being recorded from mussels in both this and neighbouring harbours (Delgado *et al.*,1990).

Alexandrium minutum was present in red tides inside the Eastern harbour of Alexandria, Egypt (Zaghloud and Halim, 1992), and along the Western coast of Turkey in May 1983 (Koray and Buyukisik, 1988). Recurrent blooms in Turkey are stated to contain the species between March and June (Koray, 1990; Koray *et al.*,1992). *Alexandrium minutum* has also been recorded along the coast of Lebanon where, however, concentrations are low and no health problems were recorded (Lakkis, 1991).

Neurotoxic shellfish poisoning (NSP), is caused by *Gymnodinium breve*, with symptoms similar to, but milder than, PSP. The motile form of the dinoflagellate produces several neurotoxins, collectively called brevetoxins.

Apart from *Gymnodinium* blooms in which individual species were not identified (see above); blooms containing the species responsible for causing NSP, *Gymnodinium breve*, are also mentioned as having been recorded in the North of Spain and in the Eastern Mediterranean (Steidinger, 1983; Berland and Bellan, 1990; Pagou and Ignatiades, 1990). Records of blooms in specific Mediterranean localities containing *Gymnodinium breve* include the Gulf of Saronikos, Greece, (Pagou, 1990).

Diarrheic shellfish poisoning (DSP), is caused by a number of toxic components isolated from shellfish associated with human symptoms characterized by diarrhoea, nausea, vomiting and abdominal pain. The algae responsible are considered to be *Dinophysis*, *Prorocentrum* and related species. In Europe, the consumption of shellfish that have filtered cells of the dinoflagellate genus *Dinophysis* have led to cases of intestinal upset. Two varieties appear responsible for DSP: *Dinophysis acuta* and *D. acuminata*. DSP is a significant problem in northern Spain, Ireland, and the Mediterranean/Adriatic Sea. The toxin in DSP appears to be okadaic acid and some related compounds, the dinophysistoxins. While at first glance, diarrhoea would appear to be a relatively minor ailment compared to symptoms of PSP and domoic acid, the DSP toxins have been reported to be tumour-promoting agents. Due to the relatively minor and generic symptoms associated with DSP, it is difficult to diagnose whether outbreaks of this particular poisoning have occurred. Up until recently, DSP was managed by mouse bioassay and/or monitoring shellfish growing waters for the presence of *Dinophysis* organisms. Within the last decade or so, chromatographic techniques using HPLC have been developed that permit both quantification and identification of the toxins. These techniques, while accurate, require well equipped laboratories with expensive equipment (i.e., HPLCs and perhaps mass spectrometers). Recently, molecular biological methods based on ELISA have been developed. For the future, these procedures offer the potential for much lower costs, rapid assessment, and also field testing. Because they have very large shellfish industries, Ireland and Spain have implemented extensive monitoring programs for DSP. Nevertheless, if monitoring costs are to be controlled, we need a far better understanding of the life cycle of *Dinophysis* and toxin production. A full understanding of the production of the toxins by *Dinophysis* has proved difficult because the culturing of this dinoflagellate in the laboratory has proved very challenging. For reasons that are not understood, the organism can only be grown through one or two generations under laboratory conditions. Until the organism can be cultured in sufficient quantities, studies on the toxins will be hampered.

Venerupin poisoning, is a non-paralytic human intoxication different from DSP, and is caused by consumption of oysters and clams which feed on toxic dinoflagellates of the genus *Prorocentrum*, mainly *Prorocentrum minimum*. The heat-stable toxin induces the rapid onset of nausea, vomiting, diarrhoea, headache and nervousness. In fatal poisoning, acute yellow atrophy of the liver, extreme excitation, delirium and coma occur.

DSP is reported as widespread in the Adriatic (Shumway, 1990), and *Dinophysis* species have been recorded in both the Northern and Central Adriatic, DSP intoxication being recorded in the former sub-region (Boni *et al.*,1992), A number of *Dinophysis* species are present in the Tyrrhenian Sea, but DSP was never detected in local shellfish (Innamorati *et al.*, 1989b).

Prorocentrum lima, the species within the genus *Prorocentrum* responsible for DSP, has been recorded from the Northwestern Adriatic (Moro and Andreoli, 1991), the Gulf of Trieste (Boni *et al.*, 1992) and the Tyrrhenian Sea (Innamorati *et al.*,1989a, 1989b). *Prorocentrum minimum*, responsible for Venerupin Poisoning, is described as recently increasing in occurrence in the Eastern Adriatic (Marasovic, 1986). There are numerous records of the species from other parts of the Adriatic (UNEP/FAO/WHO, 1995). It has also been recorded from lagoons along the French Mediterranean coast (Leveau *et al.*, 1989).

Amnesic shellfish poisoning (ASP), is caused by a toxin (Domoic acid), produced by the diatom *Nitzschia pungens*. This toxin is a mild neurological poison compared to PSP, causing gastrointestinal distress with abdominal cramps, nausea, vomiting and diarrhoea, as well as neurological symptoms involving memory loss and disorientation which can persist in severely-affected cases. In addition to dinoflagellates, a number of chlorophytes (green algae) and rhodophytes (red algae) can also be responsible for human intoxications, pathological phenomena being present in the respiratory tract in association with neurotoxic shellfish poisoning. Other biotoxins produced by cyanophytes (blue-green algae), also called cyanobacteria, cause contact dermatitis and respiratory irritation has also been described. Comprehensive reviews of aquatic biotoxins have been compiled (UNEP/FAO/WHO, 1995). *Nitzschia* species, including *Nitzschia pungens*, responsible for ASP, occurs in mucilaginous aggregates from diatoms (Viviani *et al.*,1992). This mucilage phenomenon in the Adriatic has given rise to worries over health on both sides of this Sea. The presence of domoic acid, however, was not recorded during monitoring programmes (Viviani *et al.*, 1992).

7.5 *Epidemiology of shellfish-associated illness*

Despite the variety of viruses transmitted by the faecal–oral route, many of which can be shown to contaminate shellfish or their harvesting areas, only a few have been epidemiologically linked to disease following consumption of bivalve shellfish or other and general seafood. Illness has been related to viruses causing gastroenteritis and viruses causing hepatitis.

Hepatitis A is the most serious virus infection linked to shellfish consumption causing a serious debilitating disease and even, occasionally, death. The first documented infectious hepatitis outbreak occurred in Sweden in 1955 when 629 cases were associated with raw oyster consumption (Roos, 1956). Since this time many hepatitis A virus outbreaks world-wide have been linked to bivalve mollusc consumption and have been reviewed by several authors (Richards, 1985; Rippey, 1994; Jaykus *et al.*, 1994). Occasionally such outbreaks assume an epidemic scale and are worthy of special note. The most graphic illustration being an outbreak of Hepatitis A in Shanghai, China in 1988 when almost 300000 cases were traced to the consumption of clams harvested from a sewage polluted area (Halliday *et al.*, 1991; Tang *et al.*, 1991). This outbreak ranks as the largest viral food poisoning. In the outbreak, the estimated attack rates in those who had, and had not, eaten were about 12% and 0.5% respectively with a very high overall hepatitis attack rate during the epidemic of 4083 per 100000 population (1 person involvement may only become evident through 25) (Halliday *et al.,* 1991). The USA has also reported a number of shellfish associated hepatitis A outbreaks (Richards, 1985; Rippey, 1994) as has UK (Sockett *et al.*, 1985) and other countries such as Italy (Mele *et al.*, 1989; Malfait *et al.*., 1996) and Japan (Fujiyama *et al.*, 1985). A hepatitis A epidemic in western France in 1991/1992 involving around 800 cases was considered to be associated with shellfish consumption although such studies have been published. Over 1000 cases of oyster-, raw shellfish- or clam-associated hepatitis A occurred in several US States during several major outbreaks from 1961 to exposure to a jaundiced person (Koff *et al.,* 1967). In a Japanese study between 1976 and 1985 651 cases were examined for hepatitis A risk following a contamination event (Glass *et al.,* 1996a). Of the patients with hepatitis A, ingestion of raw shellfish was the highest identified risk factor (11%) slightly exceeding general familial contact with a patient (10%). Hepatitis A virus was considered the main risk factor identified. Recent estimates suggest that shellfish may be responsible for 70% of all hepatitis A cases in Italy (Salamina and D'Argenio, 1998).

Table 17
Summary of epidemiologic characteristics of 46 outbreaks of infection throughout the world that were associated with ingestion of bivalves, by pathogen, 1969-2000

Agent	No. of outbreaks	no. of patients	Country or countries reporting outbreak	Type(s) of bivalve(s)
Calicivirus/Norwald-like viruses/small round-structures virus[b]	18[a]	5923	USA, UK, Australia, Japan, Spain	Clams, oysters
Hepatitis A virus	8	290,965[c]	USA, Italy, China, Australia	Clams, oysters, mussels
Vibrio parahaemolyticus	5	669	USA, Canada	Oysters, clams
Vibrio cholera	4	120	Malaysia, Italy, USA	"Shellfish,", "bivalves", oysters
Vibrio vulnificus	1	72	USA	Oysters
Vibrio mimicus	1	17	USA	Oysters
Vibrio hollisae	1	2	USA	Oysters
Salmonella species	3	98	Singapore, UK, Japan	Oysters, cockles
Shigella flexneri	1	40	France	Shrimps, mussels
Shigella sonnei	1	24	USA	Oysters
Plesiomonas shigelloides	2	54	USA, Canada	Roasted oysters
Listeria monocytogenes	1	4	New Zealand	Smoked mussels

[a] Includes 3 outbreaks of infection in which pathogen identification was inconclusive , but the pathogen was presumed to be a virus
[b] Small round-structures virus is also designated as "Snow mountain virus"
[c] Includes the 190,000 cases form the Shanghai outbreak

Adapted by Potasman *et al.,* 2002

7.5.1 Infectious diseases related to the consumption of seafood

Seafood – and particularly molluscs normally eaten uncooked - is a commonly implicated vehicle for the transmission of infectious diseases caused by enteric micro-organisms (including bacteria and viruses) that enter the marine environment through the disposal of urban/domestic wastewater. Pathogenic bacteria can remain viable in the sea for days to weeks, and viruses can survive in the marine environment or in the tissues of fish and seafood for months. Filter-feeding shellfish – whose breeding areas are often placed near sources of nutrients, such as wastewater outfalls or polluted estuaries – are highly prone to concentrating high levels of pathogens. Conventional depuration techniques are used to help clean shellfish harvested in contaminated waters. Shellfish are held in clean, disinfected water tanks for 36-48 hours of self cleansing. This is partially effective in removing bacterial contamination, but less effective for viruses, which are tightly adsorbed to the internal tissues of the molluscs. Thus, eating raw or lightly steamed shellfish harvested from such contaminated – but considered acceptable – marine waters can cause infection and disease in a significant percent of the exposed population.

There is firm epidemiological evidence for numerous sporadic cases – not reported as part of epidemics – of the transmission of infectious hepatitis by eating raw or lightly steamed shellfish. It was reported that some 25% of all the cases of HAV during a non-epidemic period in Boston were apparently associated with the ingestion of raw or lightly steamed shellfish. Rose and Sobsey, (1993) have written on the development of the methodology for quantitative risk assessment associated with exposure to virus contamination in shellfish. They have estimated that the risk of infection for infectious HAV for individuals who consume one raw shellfish serving of 60 grams harvested from approved waters in the United States is about 1 per 100, or 1%.

Based on reports from the Food and Agricultural Organization, it has been estimated that some eight million tons of molluscs, including clams, oysters, mussels and cockles, are harvested and marketed globally each year. Assuming that one kilogram of gross shellfish, including shells, is required for each shellfish meal or serving, Shuval (1999) has estimated that some eight billion shellfish meals are consumed globally per year.

Working with the assumption that some 88-90% come from clean safe waters and/or are not eaten raw, and using the risk of infection and disease drawn from the risk estimate study of Rose and Sobsey (1993), Shuval (1999) has estimated that each year there are about 2.5 million clinical cases of infectious hepatitis globally, with some 25,000 fatalities and 25,000 cases of long term disabilities from liver damage caused by eating contaminated shellfish.

7.5.2 Survival of enteric micro-organisms in marine waters

Several factors influence the survival of enteric micro-organisms in the marine environment. These include salinity, type of microorganism, temperature, sediments, nutrients, antagonistic factors, light, and dissolved oxygen. These characteristics have already been discussed in relation to pathogen survival in recreational waters and the reader is referred to section 2.0 for detailed discussion.

Research has demonstrated that inactivation or die-off rates for enteric micro-organisms are greater in waters of greater salinity, such as estuarine waters and seawaters, than in fresh waters. Coliforms survive poorly in marine waters, and this is one of the major reasons that this group of bacteria are inadequate predictors of the presence of pathogens. *E. coli* survival rates are more reflective of the pathogens; but at warmer temperatures the die-off rates for many of the pathogens appear to be slower than *E. coli.*

The intrinsic nature of the organism will influence the longevity of the pathogen in the marine environment. Viruses and protozoa are unable to replicate in the environment, but many of the enteric bacteria can grow under appropriate conditions of temperature and nutrients. In tropical areas, coliforms may be a part of the natural freshwater microbial flora, so that fresh water flows in the Gulf and southern Atlantic states may be contributing to the coliform levels in marine waters in the absence of any association with pathogens.

Survival of bacteria and viruses has historically been measured by cultivation techniques and may underestimate counts ten-fold (Garcia-Lara *et al.* 1991). In stressed environments such as marine waters, bacteria have been shown to remain viable even when nonculturable.

Very little information is available on the survival of protozoa in marine waters. Investigators in the 1920s and 1930s reported that *Entamoeba* survival was unaffected by salt concentrations found in seawater. *Giardia* cysts maintained the ability to encyst at the same rate for up to 12 days in seawater, surviving for 26 days at 10 to 20°C and up to 28 days in fresh waters (DeRegnier *et al.*, 1989).

DeRegnier *et al.,* 1989 demonstrated that oocysts can survive for relatively long periods in seawater at salinities and temperatures overlapping those in which oysters live. It has also been determined that oocysts could be detected in gill washings (within 72 h of exposure) of half the oysters exposed to as few as 10 oocysts and in gill washings of all those exposed to 100 or more oocysts. Finally, oocysts of *C. parvum* were recovered from naturally exposed oysters and demonstrated that they were infectious in neonatal mice. It is important to emphasize that the oysters examined in the present study were from sites selected because of their close proximity to possible sources of contamination, with three of the sites open to commercial harvesting and three closed. However, the site that yielded infectious oocysts, was an open site, and water at that site had a low coliform count within the month before oysters were collected, suggesting that a high faecal coliform count may not necessarily be a good indicator for the presence of *C. parvum.*

Temperature is perhaps one of the most important factors influencing microbial survival and has been used as the primary parameter in developing predictive models. At cooler temperatures, below 10°C, the survival of enteric pathogens is enhanced. Enteric viruses may survive for months in marine waters at low temperatures. At temperatures above 25 to 30°C, however, bacteria may be able to proliferate. Surveys of viruses in the Gulf of Mexico have demonstrated no association with detection and temperature. This implies that other factors influence the occurrence of viruses, which may or may not affect survival (i.e., infection in the community or association with sediments).

8. CONCLUSIONS

Although in the recent years, a lot of improvement was noted either in implementing monitoring programmes or providing compliance data, there is still more to be done particularly in the Eastern and Southern parts of the Mediterranean, a condition that was also highlighted in the past. In general terms, the amount of microbiological data on seawater for bathing purposes has increased in comparison with the 1984-1994 decade; however no substantial growth was noted for the shellfish growing waters.

8.1 Indicators

Any index of water quality should be directly health-related, rather than simply concerned with the degree of general pollution by sewage, i.e. water quality should be more directly linked to those components of sewage that are responsible for adverse health effects, as are pathogenic microorganisms.

For any microorganism to be used as a regulatory parameter of public health significance for seawater, it should ideally: (a) have a health basis, (b) have adequate information available with which to derive guideline values (e.g. from epidemiological investigations), (c) be sufficiently stable in waters samples for meaningful results to be obtained form analyses, (d) have a standard method of analysis, (e) be low cost to test, (f) make low demands on staff training and (g) require basic equipment that is readily available (WHO, 2003).

On the other hand, it has always been acknowledged that no individual indicator is ideal and no indicator system perfect. A certain number of comparative results obtained, have indicated some form of pathogen/indicator correlation, however in many cases statistically significant within the framework of individual studies, there is a wide range of variation between the results of different studies. Microorganisms commonly used in regulations include the following: (a) Intestinal enterococci: meet all the characteristics for a regulatory parameter of importance, (b) E. coli: is intrinsically suitable for fresh waters, but not for marine waters, (c) Total coliforms: is inadequate indicator, as they are not specific to faecal material, (d) Thermotolerant coliforms: include non-faecally derived organisms are unsuitable as regulatory parameters, (e) Salmonella: unlikely to contribute significantly to the transmission of disease via the recreational water route , because of their low infectivity and typically relatively low numbers in sewage, which when combined with their rapid inactivation in waters, particularly seawaters, suggest limited biological plausibility, (f) Enterovirus: are costly to assay and require specialized methods. Their direct health significance varies form negligible to very high (WHO, 2003).

The main purpose of monitoring bathing waters is to provide data for use as a tool in management and decision making. Most monitoring programmes sample identified waters and analyze them for the traditional faecal bacteria indicators, *E. coli*, total coliforms and faecal streptococci, in order to assess pollution status and to monitor deterioration or improvement in bathing water quality. Epidemiological studies conducted in many parts of the world have shown that faecal streptococci provide the best dose-response relationship for gastrointestinal illness and acute febrile respiratory illness in marine waters. The WHO guidelines for safe recreational water environments were derived form these studies.

Data that have been provided by the EC countries follow the indicated parameter values of the 1976 EC Directive that include total and faecal coliforms, while the rest of the Mediterranean countries besides the 1985 UNEP/WHO criteria that include faecal coliforms, use also as quality criteria total coliforms (the majority), E. coli (three countries) and faecal streptococci (four countries). It is therefore, reasonable to compare the compliance data for all the Mediterranean countries, which are based on approximately the same parameters with a slight difference in the standards.

8.2 Bathing waters quality monitoring programmes

The purpose of monitoring programmes for bathing waters quality ifs to provide data that indicate whether beaches are safe for swimming through compliance with established criteria and standards and in the case of polluted beaches, contribute towards the identification of actual and potential pollution sources, so as to enable appropriate rehabilitation measures to be implemented.

As is indicated in chapter 5, a considerable number of countries ranging from thirteen in 1996 to twenty in 2005, have implemented monitoring programmes and have submitted the data for bathing waters compliance. Figure 2 indicates that the number of countries performing monitoring programmes in the Mediterranean region increased year by year and almost all Mediterranean countries use criteria and standards for monitoring the quality of bathing waters.

The above positive trend is also noticed in the number of sampling points, where samples were collected for analysis. In fact, following a minor decrease in 1999-2000, the number of sampling points was increased from 9500 to 11600 sampling points per year. The above results confirm that every year more and more countries with an increasing number of sampling points implemented monitoring programmes. Bathing waters in the region has shown a slight improvement in quality in terms of compliance with national legislation, although it should be noted that different countries have participated each year in the survey and national legislation differs slightly from country to country.

Around 93% of bathing waters conform with the legislation, and compared with the findings of the past assessment, it shows that the general situation remains unchanged, even with the increase of sampling stations and number of data. There is still a lot to be done for achieving a compliance percentage of about 97-99%, which will provide better degree of safety to the bathers. However, a better look at the national compliance data shows that in some countries including those of the EU, the data conforming the legislation are in the range of 98-100%, indicating that the compliance percentage in the remaining countries is much less than 98-100% and therefore more efforts have to be made by those countries.

Comparing the data of the present survey with those of the previous one undertaken in 1983-1994, a positive trend is to be noticed. In fact all countries submitted monitoring data in 2005, while in the 1980s only half the countries participated in the survey. The same is true for the number of stations monitored, and due to the increase of the number of countries participating, also increased considerably. Although the overall quality has shown a very slight decline in recent years (2003-2005), in general there has been an obvious improvement in the quality of Mediterranean bathing waters since 1983. However, following the pattern of the previous years, there is a geographical imbalance in the distribution of the sampling points, the northern and western parts of the region submitting data from a greater number of sampling points than the east and the south.

8.3 Shellfish growing waters

Throughout the world, bivalve consumption has increased considerably during the past three decades. Along with this trend, infectious outbreaks caused by bivalves have been increasingly reported from almost all continents. The most commonly implicated bivalves are oysters, followed by clams. Most of the infectious syndromes produce self-limiting gastrointestinal symptoms, but a few can be fatal. Mortality has been reported in association with HAV and *V. vulnificus* infections, especially in immunosuppressed patients. People with reduced immunity are at high risk of serious disease—and possibly death—after eating raw or undercooked infected bivalves, and they should be advised to avoid this type of cuisine.

Evaluation of the studies examining the effects of growth and development on microbial contamination of coastal waters reveals many strong correlations and other general observations that help explain the influential role that humans play in coastal ecosystems. The findings have broad application, but they must also be interpreted and applied to fit the unique site characteristics that exist in all settings. Analysis of the available scientific literature points that two significant and related trends—population growth and urbanization—are stressing and degrading coastal ecosystems. Bivalve molluscan shellfish are effective carriers of enteric viruses and other pathogens. Actions that prevent and control faecal pollution in coastal areas where shellfish are grown and harvested are vital for safeguarding public health and environmental quality. Microbial contamination is chronic and pervasive in many coastal areas and is closely correlated with population densities, development levels, rainfall events, stormwater runoff, and river flows.

Watershed hydrology has a significant effect on water quality. Shellfish growing areas can be degraded at low levels of development if there are raw faecal discharges or if hydrologic processes are disrupted and there is high connectivity between the pollution sources and nearshore waters. Microbial concentrations in stormwater are generally high, due in part to significant faecal loadings from pets, other domestic animals, and wildlife.

As noticed in the survey conducted between 1983 and 1994, the lack of monitoring data on shellfish growing waters precludes an assessment, both on the current situation and on any progress effected since the 1985 assessment, which apart from only reflecting the position between 1976 and 1981 was based on an insufficient number of areas to justify regional generalization. Only a limited number of countries provided compliance monitoring data and it was the same difficult to find data from the EU countries. However, the general situation should be viewed in the light of incidence of gastrointestinal diseases and disorders, both among coastal populations and tourists, which provide cause for concern. Admittedly, overall morbidity statistics in themselves are insufficient, as practically all disease caused by pathogens are capable of being contracted through media other than the marine environment and most in fact, are probably so caused. However, this deficiency is offset in many countries by the strict quality standards imposed on shellfish destined for human consumption through public health and related legislation, that also includes surveillance and early warning systems in the EU countries.

9. RECOMMENDATIONS

For both bathing and shellfish growing waters, the discharges of human origin along with non-point sources of pollution, constitute the most common and polluting sources of seawater contamination. Sewage treatment plants are still missing from many urban areas along the coast and a percentage of urban waste disposed in the Mediterranean Sea is still untreated. Sewage should be discharged after treatment or even in enclosed or semi enclosed areas after tertiary treatment in adequately designed and well operated treatment plants. The technology is available and not expensive. The health costs and other economic losses, especially in tourist areas due to contamination of coastal waters, is much higher than the investment necessary for achieving an acceptable sewage effluent quality. In addition, in most Mediterranean countries, all types of agricultural practices and land use are treated as non-point sources of water pollution. As it is very difficult to estimate the input from these diffused sources into the Mediterranean sea quantitatively, all countries should adopt a holistic approach to water resource management, based on the integrated assessment of water quality, from the coastal waters to the entire catchment area.

A final recommendation for sewage discharges is that further data and information on water quality and the operation of sewage treatment plants needs to be available.

There is still much to be done in terms of improving bathing water quality in the Mediterranean, particularly in the south and eastern part of the region. It is likely that bathing water quality will need to be further improved as legislation is tightened. The EC bathing water Directive for example has been revised and will require higher standards of quality. It will be difficult for improvements to be made if there is not a better understanding of the sources of pollution, and in particular the balance between point source and diffuse sources. Identification of sources of indicator organisms forms an important part of water quality management allowing targeted risk management and remediation to improve water quality and protect public health.

- Although much effort has been made through the MED POL programme, there is still a scarcity of data from some regions, especially from the southern and eastern part of the Mediterranean Sea. The monitoring capabilities of some Mediterranean countries have to be improved and further strengthened in particular regarding marine pollution prevention and control mechanisms. Countries should also ensure that all relevant details of their legislation, programmes and measures taken in accordance with the provisions of the Barcelona Convention and Protocols, as well as any Resolution adopted in terms of such Convention and Protocols, are submitted to the Secretariat of the Mediterranean Action Plan and to the MED POL programme, as provided by the terms of the said Convention and Protocols.

- It is of paramount importance that in line with the new WHO Guidelines for safe bathing water quality and the recently approved EC Directive on bathing waters, new criteria and standards should be followed by the Contracting Parties, in order to implement uniform criteria for compliance monitoring and to use comparable, but not stricter standards than those of the EU. However, training courses and intercalibration exercise in microbiological methods should continue including *inter alia* data quality assurance and updating of the relevant procedures for the implementation of the legislation. In addition, capacity building assistance should be provided for sampling methods, microbiological methods of seawater analysis, good laboratory practice and the assessment and control of health risks deriving from swimming.

- It should be seriously considered that repeated monitoring throughout the summer season including sampling and analyses every fortnight requires elevated funds, especially if the country has a long shoreline with plenty of designated bathing waters. Therefore, cost effectiveness should always guide monitoring programme. A meaningful solution to such an issue is represented by preparing "beach profiles" along with sanitary inspections which allow the identification of sources of pollution and other environmental threats. In the absence of pollution sources and following a five year full monitoring programme with no negative results, a severe decrease in the number of sampling could occur, thus reducing considerably the monitoring costs and keeping the health status at the same high level.

10. REFERENCES

Amel BK, Amine B. and Amina B. (2006). Survival of *Vibrio fluvialis* in seawater under starvation conditions. *Microb Res* (in press).

Anderson, DM. (1989). Toxic algal blooms and red tides: A global perspective. In: Okaichi T, Anderson DM, Nemoto T, ed. Red tides: Biology, environmental science and toxicology. New York, NY, Elsevier Science Publishing Co., pp. 11-12.

Anderson, KL., Whitlock JE and Harwood VJ. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Appl Environ. Microb*, 71 (6), 3041-8.

Anonymous, (1983). Epidemiologic notes and reports of gastrointestinal illness among scuba divers – New York. Morbidity and Mortality Weekly Report, 32(44), 576-577.

Arean, VM. (1962) The pathgologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). *American Journal of Pathology*, 40, 393-415.

Arvanitidou, A., Constantinidis, TC. and Katsouyannopoulos, V. (1995). A survey on *Campylobacter* and *Yersinia* spp. occurrence in sea and river waters in northern Greece. *The Science of the Total Environment,* 171(1 - 3, 27), 101 - 106.

Barcina I., Lebaron P. and Vives-Rego J. (1997). Survival of allochthonous bacteria in aquatic systems: a biological approach. *FEMS, Microbiol. Ecol.* 23, 1-9.

Barwick, RS., Levy, DA., Craun, GF., Beach, MJ and Calderon, RL (2000). Surveillance for waterborne disease outbreaks – United States, 1997-1998. Mortality and Morbidity weekly Report, 49, 1-37.

Balayan, M.S. (1993) Hepatitis E virus infection in Europe: regional situation regarding laboratory diagnosis and epidemiology. *Clinical and Diagnostic Virology,* **1**(1), 1 - 9.

Bing-Mu, H., Chihpin, H., Chih-Li, LH., Yeong-Fua, H. and Yeh, JH. (1999). Occurrence of *Giardia* and *Cryptosporidium* in the Kau-Ping river and its watershed in southern Taiwan. *Water Research,* 33(11), 2701-2707.

Bogosian, G., Morris PJL and O'Neil JP. (1998). A mix culture recovery method indicates the enteric bacteria do not enter the viable non culturable state. *Appl. Environm. Microbiol.* 64, 1736-1742.

Bogosian, G., Sammons, LE., Morris PJL, O'Neil JP., Heitkamp, MA. and Weber, DB. (1996). Death of Escherichia coli K-12 strain W3110 in soil and water. *Appl. Environm Microbiol*, 62, 4114-4120.

Bonadonna L, de Mattia M, Liberti R, Volterra L. (1993). Presenza e distribuzione di stafilococchi in ambienti marini. [Presence and distribution of staphylococci in the marine environment.] *L'Igiene Moderna*, 99, 706-714.

Booth, DB. (1991). Urbanization and the natural drainage system – impacts, solutions, and prognoses. *The Northwest Environmental Journal.* (1), 93-118.

Booth, DB. and Jackson, CR. (1997). Urbanization of Aquatic Systems: Degradation Thresholds, Stormwater Detention and the Limits of Mitigation. *Journal of the American Water Resources Association*. 33(5), 1077-1090.

Borrego, JJ., Romero, P. and Mariño, F. (1991). Epidemiological study on bathers from selected beaches in Malaga. MAP Technical Reports Series, No. 53. United Nations Environment Programme, Athens.

Bremer, PJ., Obsborne CM., Kemp RA and Smith JJ. (1998). Survival of *Listeria monocytogenes* in seawater and effect of exposure on thermal resistance. J.*Appl. Microbiol.,* 85 (3), 545-53.

Brennhovd, M., Kapperud, O and Langeland, G (1992). Survey of thermotolerant Campylobacter spp. and Yersinia spp. In three surface water sources in Norway. International Journal of Food Microbiology, 15(3-4), 327-338.

Brisou, J. (1976). An environmental sanitation plan for the Mediterranean seaboard; Pollution and human health. Public Health Papers, No. 62. World Health Organization, Geneva.

Brisou, J., C. Tysset, M. Mallioux, and S. Espinasse (1962). Recherches sur les vibrions marins. A propos de 44 souches isolées de moules (*Mytilus galloprovincialis*) du littoral algérois. *Bulletin de la Societé de pathologie éxotique*, 55 :260-275

Cabelli VJ., Dufour, AP., McCabe, LJ and Levin, MA (1982). Swimming associated gastroenteritis and water quality. *American Journal of Epidemiology*, 115(4), 606-616.

Catalao Dionisio LP., Joano M., Ferreiro VS., Fidalgo ML., Garcia Rosado ME. and Borrego JJ. (2000). Occurrence of *Salmonella* spp in estuarine and coastal waters. *Antonoe Van Leuwenhoek,* 78 (1), 99-106.

Carlucci AF and Pramer D (1960). An evaluation of factors affecting the survival of Esherichia coli in sea water II: salinity, pH, and nutruents. Appl Environm Microbiol., 8, 247-250.

CDC (1997) Results of the public health response to Pfiesteria workshop - Atlanta, Georgia, September 29-30, 1997. *Mortality and Morbidity Weekly*, 46(40), 951-952.

CDR (1991) Paralytic shellfish poisoning. *Communicable Disease Report*, 1(22), 1.

CEC, EPA, WHO (2000) Monitoring Bathing Waters. A practical guide to the design and implementation of assessments and monitoring programmes

Chandram A. and Hatha Mohamed, AA. (2005). Relative survival of *Escherichia coli* and *Salmonella typhimurium* in tropical estuary. *Wat Res,* 39(7), 1397-403.

Chang, CY., Thompson, H., Rodman, N., Bylander, J and Thomas, J (1997). Pathogenic analysis of Aeromonas hydrophila septicemia. Annals of Clinical and Laboratory Science, 27(4), 254-259.

Cheung, WHS., Chang, KCK and Hung, RPS. (1990). Health effects of beach water pollution in Hong Kong. *Epidemiol. Infect.* 105, 139-162.

Clokie, MR. and Mann, NH. (2006). Marine cyanophages and light. *Environm Microb*, 8 (12), 2074-82.

Colburn, KG., Kaysner, CA., Abeyta, Jr., C. and Wekell, MM. (1990). *Listeria* species in a California coast estuarine environment. *Appl. Environ. Microbiol.* 56, 2007-2011.

Cowden, JM., Ahmed, S., Donaghy, M. and Riley, A. (2001). Epidemiological investigation of the central Scotland outbreak of *Escherichia coli* O157 infection, November to December 1996. Epidemiology and Infection, 126(3): 335-341.

Crabtree, P.A., Gerba, K.D., Rose, C.P., J.B and Haas, C.N. (1997) Waterborne adenovirus: a risk assessment. *Water Science and Technology*, 35(11 - 12), 1 - 6.

Crane, SR., Moore, JA., Grismer, ME. and Miner, JR. (1983). Bacterial pollution from agricultural sources: A review. Trans. *American Society of Agricultural Engineers,* 26(3), 858-866.

Current, WL. (1987). *Cryptosporidium:* Its biology and potential for environmental transmission. CRC *Crit. Rev. Environ. Control*, 17, 21-51.

Davies CM., Long JA., Donald M and Ashbolt NJ (1995) Survival of fecal micro-organisms in marine and freshwater sediments. App. Envirom. Microbiol., 61, 1888-1896.

De Brito, T., Beohm, GM. and Yasuda, PH. (1979). Vascular damage in acute experimental leptospirosis of the guinea-pig. *Journal of Pathology*, 128, 177 - 182.

DePaola, A., Hopkins, LH., Peeler, JT., Wentz, B. and McPhearson, RM. (1990). Incidence of *Vibrio parahaemolyticus* in U.S. coastal waters and oysters. *Appl. Environ. Microbiol.* 56:2299-2302.

DeRegnier, D.P., Cole, L., Schupp, DG., and Erlandsen, SL. (1989). Viability of *Giardia* cysts in lake, river, and tap water. *Appl. Environ. Microbiol.* 55(5):1123-1129.

Dowidart A, Abdel-Monem MH (1990) Effect of chemical pollutants on bacterial counts in El-Temsah Lake area, Ismailia, Egypt. *Journal of the Egyptian Public Health Association*, 65(3-4), 305-328.

EC (1976), Council Directive of 8th December 1975 Concerning the Quality of Bathing Water (76/160/EEC), Official Journal of the European Community, 5th February 1976, L31/1, Brussels.

EC (1979), Council directive of 30 October 1979 on the quality required of shellfish waters (79/923/EEC). *Official Journal of the European Communities,* No. L281: 47-52.

EC (1991). Council directive of the 15 July 1991 laying down the health conditions for the production and placing on the market of live bivalve mollusks (91/271/EEC). Luxembourg: Official Journal if European Communities, 1991.

EC (2006). Directive 2006/7/EC of the European Parliament and of the council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union, L64/37.

Efstratiou M., Mavridou A., Richardson S.C. and Papadakis J.A. (1997): Correlation of bacterial indicator organisms with *Salmonella spp, St. aureus* and *Candida albicans* in seawater. *Letters in Applied Microbiology* , 26, 342-346.

El Sharkawi, F. and Hassan, MNER (1982). The relation between the state of pollution in Alexandria swimming beaches and the occurrence of typhoid among bathers. *Bulletin of the High Institute of Public Health Alexandria,* 12, 337-351.

Elliot, EJ., Robins-Browne, RM., O'Loughlin, EV., Bennett-Wood, V., Bourke, J., Henning, P., Hogg, G.G., Knight, J. and Powell, H. (2001). Nationwide study of haemolytic uraemic syndrome: clinical, microbiological and epidemiological features. *Archives of Disease in Childhood*, 85(2): 125-131.

Enriquez, CE. and Gerba, CP. (1995). Concentration of enteric adenovirus 40 from tap, sea and waste water. *Water Research*, 29(11), 2554 - 2560.

Esrey, SA., Feacham, RG and Hughes, JM (1985). Interventions for the control of diarrhoeal disease among young children: improving water supplies and excreta disposal facilities. Bulletin of the World Health Organisation, 63(4), 757-772.

EU, (2006). DIRECTIVE 2006/7/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union, L64/37.

Eyles, R., Niyogi, D., Townsend, C., Benwell, G and Weinstein, P. (2003). Spatial and temporal patterns of Campylobacter contamination underlying public health risk in the Taieri River, New Zealand. Journal of Environmental Quality, 32, 1820-1828.

FAO/UNESCO/IOC/WHO/WMO/IAEA/UNEP (1983) Coordinated Mediterranean Pollution Monitoring and research programme (MED POL Phase I) – Programme Description. UNEP Regional Seas Reports and Studies No. 23. United Nations Environment Programme, Geneva.

Fattal, B., Peleg-Olevsky Agurshy, T. and Shuval, H.L. (1987). The association between sea water pollution as measured by bacterial indicators and morbidity of bathers at Mediterranean beaches in Israel. *Chemosphere*, 16, 565-70

Fattal, B. and Shuval, HI (1989). Epidemiological research on the relationship between microbial quality of coastal seawater and rotavirus induced gastroenteritis among bathers on the Mediterranean Israeli beaches. Research project No. ICP/CEH 039-ISR 16 (D0. World Health Organization Regional Office for Europe, Mediterranean Action Plan, Athens.

Feachem, RG., Bradley, DH., Garelick, H. and Mara, DD. (1983). Sanitation and Disease Health Aspects of Excreta and Wastewater Management. New York: John Wiley and Sons.

Ferley, JP., Zmirou, D., Balducci, F., Baleux, B., Fera, P., Larbaigt, G., Jacq, E., Moissonnier, B., Blineau, A. and Boudot, J. (1989). Epidemiological significance of microbiological pollution criteria for river recreational waters. International Journal of Epidemiology, 18(1), 198-205.

Fewtrell, L., Godfree, A., Jones, F., Kay, D., Salmon, RL. and Wyer, MD. (1992). Health effects of white-water canoeing. *Lancet* 339:1587-1589.

Fewtrell, L., Godfree, A.F., Jones, F., Kay, D., Salmon, R.L. and Wyer, M.D. (1994). Pathogenic micro-organisms in temperate environmental waters. Samara Publishing, Cardigan, Dyfed, UK.

Fleisher, J., Jones, F., Kay, D., Stanwell-Smith, R., Wyer, MD and Morano, R. (1993). Water and non-water related risk factors for gastroenteritis among bathers exposed to sewage contaminated marine waters. International Journal of Epidemiology, 22, 698-708.

Fleisher, J., M., Kay, D., Salmon, RL., Jones, F., Wyer, MD. and Godfree, AF (1996). Marine waters contaminated with domestic sewage: nonenteric illnesses associated with bather exposure in the United Kingdom. *Am. J. Public Health,* 86, 1228–1234.

Foulon, G., Maurin, J., Quoi, NN and Martin-Bouyer, G. (1983). Etude de la morbidite humaine en relation avec la pollution bacteriologique des eaux de baignade en mer. *Revue Francaise des Sciences de l'eau,* 2(2), 127-143.

Fournier, S., Dubrou, S., Ligoury, O., Gaussin, F., Santillana-Hayat, M., Sarfati, C., Molina, JM. and Derouin, F. (2002) Detection of *Microsporidia*, *Cryptosporidia* and *Giardia* in swimming pools: a one-year prospective study. *FEMS Immunology and Medical Microbiology,* **33**, 209 - 213.

Gannon, JJ., and Busse, MK. (1989). *E. coli* and enterococci levels in urban water and chlorinated treatment plant effluent. *Water Res. Journal,* 23, 1167-1176.

Garcia-Lara, J., Menon, P., Servais, P. and Billen, G. (1991). Mortality of faecal bacteria in seawater. *Appl. Environ. Microbiol.* 57, 885-888.

Gonzales JM., Iriberri J., Egea L and Barcina I (1992): Characterisation of culturability protstan gazing and deth of enteric bacteria in aquatic ecosystems. Appl. Environm Microbiol., 58, 998-1004

Gourmelon M., Touati D., Pommepuy M and Cornier M (1997). Survival of *Escherichia coli* exposed to visible light in seawater: analysis of rpoS-dependent effects. *Can J Microbiol*, 43 (11) 1036-43.

Grimes, DJ. and Colwell, RR. (1986). Viability and virulence of *Escherichia coli* suspended by membrane chamber in semitropical ocean water. *FEMS Micro. Lett.* 34, 161-165.

Haas CN, Rose JB, Gerba CP. (1999). Quantitative microbial risk assessment. New York, John Wiley & Sons, Inc., 449 pp.

Haas, CN., Rose, JB and Gerba, CP (1990). Quantitative microbial risk assessment. John Wiley and Sons, Inc, New York, United States.

Hallegraeff GM (1993). A review of harmful algal blooms and their apparent global increase. *Phycologia*, 32(2), 77-99.

Heller R., Holler C., Sussmuth R. and Grundermann KO. (1998). Effect of salt concentration and temperature on survival of *Legionella pneumophila*. *Lettr Appl. Microbiol.*, 26 (1), 64-8.

Hernroth, BE., Conden-Hansson, A., Rehnstam-Holm, A., Girones, R. and Allard, AK. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the Blue Mussel, *Mytilus edulis*: the First Scandinavian Report. *Applied and Environmental Microbiology*. 68(9), 4523-4533.

Hielm S., Hyytia E., Andersin AB. and Korkeala H. (1998). A high prevalence of Clostridium botulinum type E in Finnish freshwater and Baltic Sea sediment samples. *J. Appl Microbiol.*, 84 (1), 133-7.

Hlady, G.W. and Klontz, K. (1996). The epidemiology of vibrio infections in Florida, 1981-1993. *Journal of Infectious Diseases*, 173, 1176-1183.

Ho, BSW. and Tam, TY. (1998). Occurrences of *Giardia* cysts in beach water. *Water, Science and Technology,* 38(12), 73-76.

Holden, WS. (1970). Water Treatment and Examination. J & A Churchill, London, UK, 248 - 269.

Holland, AF., Sanger, DM., Gawle, CP., Lerberg, SB., Santiago, MS., Riekerk, GHM., Zimmerman, LE. and Scott, GI. (2004). Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *Journal of Experimental Marine Biology and Ecology.* 298(2), 151-178.

Holland, AF., Riekerk, GH., Lerberg, SB., Zimmerman, LE. and Sanger, DM. (1998). Assessment of the Impact of Watershed Development on the Nursery Functions of Tidal Creek Habitats. In: *Workshop Proceedings: Biological Habitat Quality Indicators for Essential Fish Habitat*, July 14-15, 1997. Charleston, South Carolina. S. Ian Hartwell, ed. http://www.epa.gov/ednnrmrl/publish/book/epa-600-r-99-029/)

Hugues, B., Pietri, C., Puel, D., Crance, J.M., Cini, C. and Deloince, R. (1988). Research of enterovirus, hepatitis A virus in a bathing area over a six-month-period and their salubrity impact. *Zentralblatt Fur Bakteriologie Mikrobiologie und Hygiene*, 185(6), 560-568.

Hunter, PR. (1998). Waterborne Disease Epidemiology and Ecology. Wiley & Sons Ltd. Chichester, UK.

Jaykus, L., Hemard, M., Sobsey, M. (1994). Human enteric pathogenic viruses. In: Hackney C, Pierson M, eds. Environmental indicators and shellfish safety. New York: Chapman and Hall, 1994:92–153.

Jeng HC., England AJ. and Bradford, HB. (2005). Indicator organisms associated with the stormwater suspended particles and estuarine sediment. *Environ Sci Health*, *A tox Hazard Subst . Environ Eng.* 40 (4), 779-91.

Jones, K., Betaieb, M and Telford, DR (1990). Seasonal variation of thermophilic campylobacters in sewage sludge. Journal of Applied Bacteriology, 69, 185-189.

Johnson, DC., Reynolds, KA., Gerba, CP., Pepper, IL. and Rose, JB. (1995). Detection of *Giardia* and *Cryptosporidium* in marine waters. *Water Sci. Technol.* 31:439–442.

Johnson, DC., Enrique, CE., Pepper, IL., Davis, TL., Gerba, CP., and Rose, JB. (1997). Survival of *Giardia, Cryptosporidium*, Poliovirus and *Salmonella* in marine waters. *Water Science and Technology,* 35(11 - 12), 261 – 268

Kay, D., Fleisher, JM., salmon, RL., Wyer, MD., Godfree, AF., Zelenauch-Jacquotte, Z., Shore, R. (1994). Predicting likelihood o gastroenteritis from sea bathing; results from randomized exposure. Lancet, 344(8927): 905-909.

Kay D., Stapleton CM., Wyer MD., McDonald AT., Crowther J., Paul N., Jones K., Francis C., Watkins J., Wilkinson J., Humphrey N., Lin B, Yang L., Falconer R.A. and Gardner S. (2005). Decay of intestinal enterococci concentrations in high- energy estuarine and coastal waters: towards real-time T90 values for modelling faecal indicators in recreational waters. *Wat Res* , 39 (4), 655-667.

Kay, D., Fleisher, J., Wyer, M., Salmon, RL. (2001). Re-analysis of the sea bathing data from the UK randomized trial. A report to DETR. Aberystwyth, University of Wales, Centre for Research in the Environment and Health, 17pp.

Keene, WE., McAnulty, JM., Hoesly, FC., Williams, LP., Hedber, K., Oxman, GL., Barrett, TJ., Pfaller, MA., Fleming, DW. (1994). A swimming associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonneri. New England Journal of Medicine*, 331, 579-584.

Knight, IT., Shults, S., Kaspar, CW and Colwell, RR. (1990). Direct detection of *Salmonella* spp. in estuaries by using a DNA probe. *Appl. Environ. Microbiol.* 56, 1059-1066.

Konowalchuk, J., Spiers, I and Satvric, S. (1977). Vero response to a cytotoxin of Escherichia coli. Infection and Immunity, 18, 775-779.

Kocasoy G. (1989). The relationship between coastal tourism, sea pollution and public health; a case study from Turkey. *The Environmentalist*, 9(4), 245-251.

Kramer, MH., Sorhage, FE., Goldstein, ST., Dalley, E., Wahlquist, SP., Herwaldt, BL. (1998). First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake. *Clinical Infectious Diseases*, 26, 27-33.

Lambert, WCA., van Breemen, H., Ketelaars, AM., Hoogenboezem, W. and Medema, G. (1998). Storage reservoirs-a first barrier for pathogenic micro-organisms in The Netherlands. *Water Science and Technology,* 37(2), 253-260.

Lansbury and Ludlam, (1997). O157:H7: lessons from the past 15 years. Journal of Infection, 34, 189-193.

Le Chevallier, M.W., Norton, W.D. and Lee, R.G. (1991) *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. *Applied Environmental Microbiology,* **57,** 2617 - 2621.

Lee, JV. and Joseph, C. (2002). Guidelines for investigating single cases of legionnaires' disease. *Communicable Disease and Public Health*, 5(2), 157 - 162.

Leecaster, M. K. and Weisberg, SB (2001). Effect of sampling frequency on shoreline microbiology assessments. *Marine Pollution Bulletin,* 42, 1150-1154.

Lees, D. (2000). Viruses and bivalve shellfish. *International Journal of Food Microbiology*, 59, 81–116.

Lehane, L. (2000). Paralytic shellfish poisoning: A review. National Office of Animal and Plant Health, Agriculture, Fisheries and Forestry. Canberra, Australia.

Lerberg, SB., Holland, AF. and Sanger, DM. (2000). Responses to tidal creek macrobenthic communities to the effects of watershed development. *Estuaries*. 23(6), 838-853.

Lilja, J. and Glasoe, S. (1993). Uses and Limitations of Coliform Indicators in Shellfish Sanitation Programs. *Puget Sound Notes*. Puget Sound Water Quality Authority. Olympia, Washington.30:4-6.

Lipp, EK. and Rose, JB. (1997). The role of seafood in foodborne diseases in the United States of America. *Review of Scientific Techniques, Office International des Epizooties Review*. 16(2), 620-40.

Lipp, EK., Kurz, R. Vincent, R., Rodriquez-Palacios, C., Farrah, SR. and Rose, JB. (2001b). The Effects of Seasonal Variability and Weather on Faecal Pollution and Enteric Pathogens in a Subtropical Estuary. *Estuaries*. 24(2), 266-276.

Lipp, EK., Farrah, SA. and Rose, JB. (2001a). Assessment and impact of microbial faecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin*. 42(4), 286-293.

Maalej S., Denis M. and Dukan S. (2004). Temperature and growth-effects on *Aeromonas hydrophila* survival in natural seawater microcosms: role of protein synthesis and nucleic acid content on viable but temporarily non- culturable response. *Microbiology*, 150 (Pt 1), 181-7.

Mallin, MA. and Lewitus, AJ. (2004). The importance of tidal creek ecosystems. *Journal of Experimental Marine Biology and Ecology*. 298(2), 145-149.

Mallin, MA., Burkholder, JM., Cahoon, LB. and Posey, MH. (2000b). North and South Carolina Coasts. *Marine Pollution Bulletin*. 41(1-6), 56-75.

Mallin, MA., Williams, KE., Esham, EC and Lowe, RP. (2000a). Effect of human development on bacteriological water quality in coastal watersheds. *Ecological Applications*, 10(4), 1047-1056.

Mallin, MA., Ensign, SH., McIver, MR., Shank, GC. and Fowler, PK. (2001). Demographic, landscape, and meteorological factors controlling the microbial pollution of coastal waters. *Hydrobiologia*. 460, 185-193.

Mandilara G., Smeti E., Mavridou A., Lambiri M., Vatopoulos A. and Rigas F. (2006). Correlation between bacterial indicators and bacteriophages in sewage sludge. *FEMS Microb Lett*, 263, 119-126.

Marchman, GL. (2000). *An Analysis of Stormwater Inputs to the Apalachicola Bay*. Northwest Florida Water Management District Water Resources Special Report 00-01. Northwest Florida Water Management District. Havana, Florida. 63 pp.

Marco-Noales E., Biosca E.G., Rojo C and Amaro C. (2004). Influence of aquatic microbiota on the survival in water of the human and eel pathogen *Vibrio vulnificus* serovar E. *Environm Microbiol.*, 6 (4) 364-76.

Mary I., Tu C.J., Grossman A. and Vaulot D. (2004). Effects of high light on transcripts of stress-associated genes for the cyanobacteria Synechocystis sp. PCC 6803 and Prochloriococcus MED4 and MIT9313. *Microbiology*, 150 (Pt5), 1271-81.

Medema, GJ., van Asperen, IA., Klokman-Houweling, JM., Nooitgedagt, A., van de Laar, W. and Havelaar, AH. (1995). The relationship between health effects in triathletes and microbiological quality of freshwater. *Water Science and Technology*, 31(5 - 6), 19 - 26.

Miyagi K., Omura K., Ogawa A., Hanafusa M., Nakano Y., Morimatzu S. and Sano K. (2001). Survival of Shiga toxin producing *Escherichia coli* O157 in marine water and frequent detection of the Shiga toxin gene in marine samples form an estuary port. *Epidemiol Infect.*, 126 (1), 129-133.

Montanari, M.P., Pruzzo, C., Pane, L. and Colwell, R.R. (1999) Vibrios associated with plankton in a coastal zone of the Adriatic Sea (Italy). *FEMS Microbiology Ecology,* **29**(3), 241 - 247

Mujeriego, R., Bravo, JM and Feliu, MT (1982). Recreation in coastal waters, public health implications. Viemes Journees Etud. Pollutions, Cannes, *CIESM,* 585-594.

Mujeriego, R., J. M. Bravo, and M. T. Feliu. (1983). Recreation on coastal waters: public health implications, p. 585-594. Workshop on pollution of the Mediterranean. International Commission for the Scientific Exploration of the Mediterranean Sea, Monaco.

Munro, PM., Clement, RL., Flatau, GN. and Gautier MJ. (1994). Effect of thermal, oxidative, acidic, osmotic or nutritional stresses on subsequent culturability of *Escherichia coli* in seawater. *Microb. Ecol.* 27, 57-63.

Munro, PM., Flatau GN., Clement RL., and Gautier MJ. (1995). Influence of Rpos (KatF) sigma factor on maintenance of viability and culturability of *Escherichia coli* and *Salmonella typhimurium* in seawater. *Appl. Environm. Microbiol*, 61, 1853-1858.

National Research Council. (1999). *From Monsoons to Microbes: Understanding the Ocean's Role in Human Health.* Committee on the Ocean's Role in Human Health, National Research Council. National Academy Press. Washington, D.C. 144 pp.

National Research Council (2004). *Indicators for Waterborne Pathogens.* Committee on Indicators for Waterborne Pathogens, National Research Council. National Academy Press. Washington, D.C. 236 pp.

Noble; RT. and Fuhrman, JA. (2001). Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: Low correlation to bacterial indicators. *Hydrobiologia,* 460(1-3), 175- 184.

Noble, RT., Moore, DF., Leecaster, MK., McGee, CD and Weisberg, SB. (2003a). Comparison of total coliform, faecal coliform, and Enterococcus bacterial indicator response for ocean recreational water quality testing. *Water Research,* 37, 1637-1643. (available at ftp://ftp.sccwrp.org/pub/download/PDFs/383_micro_indicator.pdf)

Noble, RT., Dorsey, JH., Leecaster, M., Orozco-Borbón, V., Reid, D., Schiff, K. and Weisberg, SB. (2000a). A regional survey of the microbiological water quality along the shoreline of the Southern California Bight. *Environmental Monitoring and Assessment.* 64:435-447.

Noble, RT., Leecaster, MK., McGee, CD., Moore, DF., Orozco-Borbon, V., Schiff, K., Vainik, PM and Weisberg, SB. (2000b). *Storm Event Shoreline Microbiology: Southern California Bight 1998 Regional Monitoring Program Volume 3.*

Noble RT., Lee IM., Schiff KC (2004). Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. J. Appl. Microbiol., 96(3) 464-72.

Obiri-Danso, K. and Jones, K. (2000). Intertidal sediments as reservoirs for hippurate negative campylobacters, salmonellae and faecal indicators in three EU recognised bathing waters in north west England. *Water Research*, 34(2), 519-527.

Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, DuPont HL (1999). Virulence of three distinct Cryptosporidium parvum isolates for healthy adults. *Journal of Infectious Diseases*, 180, 1275-1281.

Papadakis JA., Mavridou, A., Richardson, SC., Lambiri, M. and Marcelou, U. (1997). Bather related microbial and yeast populations in sand and seawater. *Water Research*, 31(4), 799-804.

Papapetropoulou, M. and Sotiracopoulou, S. (1995). Effect of bathing on human skin flora. MAP Technical Reports Series, No. 93, pp 23-31. United Nations Environment Programme, Athens.

Paszko-Kolva J, Shahamat M and Colwell R.R. (1993). Effect of temperature on the survival of Legionella pneumophila in the aquatic environment. *Microb Releases*, 2 (2), 73-9.

Paul, MJ. and Meyer, JL. (2001). Streams in the urban landscape. *Annual Review of Ecology and Systematics*. 32, 333-365.

Paz, S., Bisharat, N., Paz, E., Kdar O. and Cohen, D (2007). Climate change and the emergence of Vibrio vulnificus disease in Israel. *Environ Res.,* 103(3), 390-6.

Perales, I. and A. Audicana. A. (1989). Semisolid media for isolation of *Salmonella spp.* from coastal waters. *Appl. Environ. Microbiol.* 55, 3032-3033.

Pitt, R., Maestre, A., Morquecho, R., Brown, T. Schueler, T., Cappiella, K., Sturm, P. and Swann, C. (2004). *Research Progress Report: Findings from the National Stormwater Quality Database*. Center for Watershed Protection. Ellicott City.

Poff, NL., Allan, JD., Bain, MB., Karr, JR., Prestegaard, KL., Richter, BD., Sparks, RE. and Stromberg, JC. (1997). The Natural Flow Regime: A Paradigm for River Conservation and Restoration. *BioScience,* 47(11), 769-784.

Polo, F., Figueras, M.J., Inza, I., Sala, J., Fleisher, J.M. and Guarro, J. (1998). Relationship between presence of Salmonella and indicators of faecal pollution in aquatic habitats. *FEMS Microbiology Letters,* 160(2), 253 - 256.

Pommepay M., Butin M., Derrien A., Gourmelon M., Colwell R.R. and Cormier M 91996): Retention of enteropathogenicity by viable but non culturable E.coli exposed to seawater and sunlight. Appl.Environm.Microbiol, 62, 1888-1896.

Pond, K. (2005). Water recreation and disease. Plausibility of associated infections: acute effects, sequelae and mortality. IWA Publishing and WHO.

Pond, KR., Rangdale, R., Meijer, WG., Brandao, J., Falcao, L., Rince, A., Masteron, B., Greaves, J., Gawler, A., McDonnell, E., Cronin, AA and Pedley, S. (2004). Workshop Report: Developing pollution source tracking for recreational wand shellfish waters. *Environmental Forensics*, 5, 237-247.

Proctor, ME and Davis, JP (2000). Escherichia coli O157:H7 infections in Wisconsin, 1992-1999. WMJ: Official Publication of the State Medical Society of Wisconsin, 99(5), 32-37.

Pruss, A (1998). A review of epidemiological studies from exposure to recreational water. *International Journal of Epidemiology*, 27, 1-9.

Rippey S. (1994). Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev,* 7, 419–425.

Robertson, LJ., Cambell AT. and Smith HV. (1992). Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Appl. Environm. Microbiol*, 58 (11), 3494-500.

Rose, JB, Epstein, PR., Lipp, EK., Sherman, BH., Bernard, SM. and Patz, JA. (2001b). Climate variability and change in the United States: Potential impacts on water- and foodborne diseases caused by microbiologic agents. *Environmental Health Perspectives*. 109(2), 211-220.

Rose, JB., Atlas, RM., Gerba, CP., Gilchrist, MJR., LeChevallier, MW., Sobsey, MD., Yates, MV., Cassell, GH and Tiedje, JM. (1999). *Microbial Pollutants in our Nation's Water: Environmental and Public Health Issues*. American Society for Microbiology. Washington, D.C. 16 pp.

Rose, JB., and Sobsey, (1993). MD. undated. Quantitative risk assessment for viral contamination of shellfish and coastal waters. Submitted to Journal of Food Protection.

Roth, NE., Allan, JD and Erickson, DL. (1996). Landscape influences on stream biotic integrity assessed at multiple spatial scales. *Landscape Ecology*. 11(41), 141-156.

Rowe, PC., Orrbine, E., Wells, GA and McLaine, PN (1991). Epidemiology of haemolytic-uraemic syndrome in Canadian children from 1986 to 1988. The Journal of Pediatrics, 119(2), 218-224.

Rozen, Y. and Belkin, S. (2001). Survival of enteric bacteria in seawater. FEMS Microbiol Reviews, 25 (5), 513-529.

Rozen, Y., Van Dyck, T.K., La Rossa, R.A. and Belkin, S. (2001). Seawater activation of *Escherichia coli* gene promoter elements: dominance of rpoS control. *Microb. Ecol.,* 42, 635-643.

Sair, AI., D'Souza, DH and Jaykus, LA. (2002). Human enteric viruses as causes of foodborne disease. Comprehensive Reviews in Food Science and Food Safety. *Institute of Food Technologies*. 1(2):73-79.

Salvatore, S., Grillo, C., Mattei, D. and Bruno, M (2006). SPME-HS/GC-MS analyses of *Ostreopsis ovata* bloom in 2005 (Genoa, Italy). ISS Publicazioni, Rapporti ISTISAN 06/9.

Schroeder, ED., Stallard, WM., Thompson, DE., Loge, FJ., Deshussess, MA. and Cox, HHJ. (2002). *Management of Pathogens Associated with Storm Drain Discharge: Results of Investigations of the Presence of Human Pathogens in Urban Storm Drains*. Report No. CTSW-RT-02-025 prepared for Division of Environmental Analysis, California Department of Transportation. Sacramento, California. 99 pp.

Shuval, H. (2003). Estimating the global burden of thalassogenic diseases: human infectious diseases caused by wastewater pollution of the marine environment. *Journal of Water and Health*, 1(2), 53-64.

Shuval, HI., (1986). Thalassogenic diseases. UNEP Regional Seas Reports and Studies No. 79. United Nations Environment Programme, Geneva

Sinton LW., Finlay RK. and Lynch PA. (1999). Sunlight inactivation of faecal bacteriophages and bacteria in sewage-polluted seawater. *Appl Environm Microbiol.*, 65 (8), 3605-13.

Smayda TJ (1989a). Primary production and the global epidemic of phytoplankton blooms in the sea: A linkage. In: Cosper EM, Bricelj VM, Carpenter EJ, ed. Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms. Berlin, Springer Verlag, pp. 449-483.

Smayda TJ (1989b). Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic. In: Graneli E, Sundstršm B, Edler L, Anderson DM, ed. Toxic marine phytoplankton. New York, NY, Elsevier Science Publishing Co., pp. 29-40.

Teunis PFM, Nagelkerke NJD, Haas CN (1999). Dose response models for infectious gastroenteritis. *Risk Analysis*, 19: 1251-1260

Teunis PFM, van der Heijden OG, van der Giessen JWB, Havelaar AH (1996). The dose-response relation in human volunteers for gastro-intestinal pathogens. Bilthoven, RIVM.

Torregrossa *et al.*, (1994).

UNEP (1978) Mediterranean Action Plan and the Final Act of the Conference of Plenipotentiaries of the Coastal States of the Mediterranean Region for the protection of the Mediterranean Sea. United Nations. New York.

UNEP (1980) Conference of Plenipotentiaries of the Coastal States of the Mediterranean Region for the Protection of the Mediterranean Sea against Pollution from Land-based Sources, May 1980. Final Act and Protocol. United Nations, New York.

UNEP (1981) Report of the Second Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols, Cannes, 2-7 March 1981, Document UNEP/IG.23/11, United Nations Environment Programme, Geneva.

UNEP (1983) Long-term Programme of Pollution Monitoring and Research in the Mediterranean. UNEP Regional Seas Reports and Studies No. 28. United Nations Environment Programme, Geneva.

UNEP (1985). Report of the Fourth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols. Genoa, 9-13 September 1985. Document UNEP/IG.56/5. United Nations Environment Programme, Athens.

UNEP (1987) Report of the Fifth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related protocols. Athens, 8-11 September 1987. Document UNEP/IG.74/5. United Nations Environment Programme, Athens.

UNEP (1989) Evaluation of MED POL Phase II monitoring data: Part II – Microorganisms in coastal areas. Document UNEP(OCA)MED WG.5/Inf.4. United Nations Environment Programme, Athens.

UNEP (1991) Report of the Seventh Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols. Cairo, 8-11 October 1991. Document UNEP(OCA)MED IG.2/4. United Nations Environment Programme, Athens.

UNEP (1995a) Report of the Ninth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols. Barcelona, 5-8 June 1995. Document UNEP(OCA)MED IG.5/16. United Nations Environment Programme, Athens.

UNEP (1995b) Final Act of the Conference of Plenipotentiaries on the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols. Barcelona, 9-10 June 1995. Document UNEP(OCA)MED IG.6/7. United Nations Environment Programme, Athens.

UNEP (1996) Final Act of the Conference of Plenipotentiaries on the Amendment of the Protocol for the Protection of the Mediterranean Sea against Pollution from land-based Sources, Syracuse, 6-7 March 1996. Document UNEP(OCA)MED IG.7/4. United Nations Environment Programme, Athens.

UNEP/MAP/WHO (2004). Municipal wastewater treatment plants in Mediterranean coastal cities (II). MAP Technical Reports Series No. 157. UNEP/MAP, Athens.

UNEP/MAP/WHO (1996). Assessment of the state of microbiological pollution of the Mediterranean sea. MAP Technical Reports Series no 108, Athens.

UNEP/WHO (1985) Assessment of the present state of microbial pollution in the Mediterranean Sea and proposed control measures. Document UNEP/WG.118/6. United Nations Environment Programme, Athens.

UNEP/WHO (1987) Assessment of the state of microbial pollution of shellfish waters in the Mediterranean Sea and proposed measures. Document UNEP/WG.160/10. United Nations Environment Programme, Athens.

UNEP/WHO (1991) Assessment of the state of pollution in the Mediterranean Sea by pathogenic microorganisms. Document UNEP(OCA)/MED WG. 89/Inf.5. United Nations Environment Programme, Athens.

US EPA, (1999) Action Plan for beaches and recreational waters. Available online. http://www.epa.gove/ORD/publications.

Van Dolah, RF, Chestnut, DE. and Scott, GI. eds. (2000). *A Baseline assessment of environmental and biological conditions in Broad Creek and the Okatee River, Beaufort County, South Carolina*. Prepared by South Carolina.

Vasconcelos, J. (2001). Factors affecting the survival of viruses in marine sediment and seawater. *Puget Sound Notes*. 45, 9-12.

Vernberg, FJ., Vernberg, WB., Porter, DE., Chandler, GT., McKellar, HN., Scott, G., Siewicki, T., Fulton, M., Bushek, D., Tufford, D. and Wahl, M. (1999). Coastal development impacts on land-coastal waters. In: *Land Ocean Interactions: Managing Coastal Ecosystems*. E. Ozhan, ed. Fourth International Conference on the Mediterranean Coastal

10. APPENDIX

ASSESSMENT OF THE STATE OF MICROBIOLOGICAL POLLUTION OF THE MEDITERRANEAN SEA

MAP Technical Series No. 108

TABLE 5.1.4

SUMMARY OF MONITORING DATA ON THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS SUBMITTED BY THIRTEEN MEDITERRANEAN COUNTRIES WITHIN THE FRAMEWORK OF THE MONITORING COMPONENT OF MED POL PHASE II, 1983-1994

A. GLOBAL DATA

Year	Number of countries	Countries submitting monitoring data	Number of sampling stations	Number of samples analyzed
1983	5	ISR, MAT, MOR, TUR, YUG	139	1358
1984	6	ISR, LEB, MAT, MOR, TUR, YUG	183	2035
1985	7	CYP, ISR, LEB, MAT, MOR, TUR, YUG	344	2717
1986	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	352	3903
1987	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	353	3908
1988	7	ALG, CYP, ISR, LEB, MAT, MOR, YUG	354	4326
1989	9	ALG, CYP, EGY, ISR, LEB, MAT, MOR, TUN, YUG	414	5041
1990	7	ALG, CYP, EGY, ISR, MAT, MOR, YUG	376	4725
1991	6	CYP, EGY, ISR, MAT, SYR, YUG	389	5112
1992	5	ALB, CRO, CYP, SYR, TUN	404	5006
1993	2	ALB, CYP	195	2786
1994	2	ALB, SYR	43	149
	13			41065

TABLE 5.1.5

COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF RECREATIONAL WATERS IN THIRTEEN MEDITERRANEAN COUNTRIES, 1983 - 1992, ACCORDING TO THE INTERIM CRITERIA ADOPTED BY THE CONTRACTING PARTIES IN 1985, SHOWING COMPLIANCE WITH INDIVIDUAL PARAMETERS , EXPRESSED AS PERCENTAGES OF TOTAL NUMBER OF STATIONS MONITORED

Year	Number of countries submitting data	Number of Stations monitored	Number of stations complying with			Number of stations complying with FC50 + FC90 only	Number of satisfactory stations
			Frequency	FC50	FC90		
1983	5	139	56 (40.3%)	128 (92.1%)	128 (92.1%)	127 (91.4%)	50 (36.0%)
1984	6	183	97 (53.0%)	165 (90.2%)	165 (90.2%)	159 (86.9%)	85 (46.4%)
1985	7	344	118 (34.3%)	316 (92.0%)	311 (90.4%)	307 (89.2%)	99 (28.8%)
1986	7	352	220 (62.5%)	304 (86.4%)	304 (86.4%)	297 (84.4%)	191 (54.3%)
1987	7	353	241 (68.3%)	314 (89.0%)	318 (90.1%)	300 (85.0%)	221 (62.6%)
1988	7	354	256 (72.3%)	322 (91.0%)	315 (89.0%)	308 (87.0%)	227 (64.1%)
1989	9	414	275 (66.4%)	369 (89.1%)	362 (87.4%)	353 (85.3%)	248 (59.9%)
1990	7	376	286 (76.1%)	366 (97.3%)	367 (97.6%)	354 (94.1%)	263 (69.9%)
1991	6	389	285 (73.3%)	348 (89.5%)	342 (87.9%)	331 (85.1%)	277 (71.2%)
1992	5	404	269 (66.6%)	346 (85.6%)	289 (71.5%)	279 (69.1%)	208 (51.5%)

TABLE 5.1.6

COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS IN EC MEDITERRANEAN MEMBER STATES, 1991 - 1994, ACCORDING TO THE GUIDE STANDARDS OF THE 1976 EC BATHING WATER DIRECTIVE FOR (A) COLIFORMS AND (B) FAECAL STREPTOCOCCI, EXPRESSED AS PERCENTAGES OF TOTAL NUMBERS OF IDENTIFIED BATHING AREAS

(Compiled from European Commission report, 1995a)

Country	Year	Identified bathing areas	Stations conforming with coliform guide standards TC80 + FC80	Stations conforming with streptococci guide standards FS90
France	1991 1992 1993 1994	1556 1934 1856 1870	1053 (67.7%) 1129 (58.4%) 1201 (64.7%) 1298 (69.4%)	1145 (73.6%) 1184 (61.2%) 1305 (70.3%) 1285 (68.7%)
Greece	1991 1992 1993 1994	1097 1203 1250 1282	932 (85.0%) 1142 (94.9%) 1170 (93.6%) 1167 (91.0%)	- 1166 (96.9%) 1246 (99.7%) 1258 (98.1%)
Italy	1991 1992 1993 1994	3824 4033 4288 4543	3205 (83.8%) 3444 (85.4%) 3516 (82.0%) 3680 (81.0%)	3425 (89.6%) 3690 (91.5%) 3964 (92.4%) 4229(93.1%)
Spain	1991 1992 1993 1994	1303 1335 1405 1490	890 (68.3%) 980 (73.4%) 1121 (79.8%) 1243 (83.4%)	574 (44.1%) 743 (55.7%) 1091 (77.7%) 1153 (78.0%)

TABLE 5.1.7

OVERALL COMPARATIVE EVALUATION OF THE MICROBIOLOGICAL QUALITY OF COASTAL RECREATIONAL WATERS OF EC MEDITERRANEAN MEMBER STATES, 1991 - 1994, IN TERMS OF COMPLIANCE WITH EC 1976 BATHING WATER DIRECTIVE (A) MANDATORY STANDARDS FOR COLIFORMS, (B) GUIDE STANDARDS FOR COLIFORMS AND (C) GUIDE STANDARDS FOR FAECAL STREPTOCOCCI, EXPRESSED AS PERCENTAGES OF TOTAL NUMBER OF IDENTIFIED BATHING AREAS

(Compiled from European Commission report, 1995a)

Year	Identified bathing areas	Stations conforming with coliform mandatory standards TC95 + FC95	Stations conforming with coliform guide standards TC80 + FC80	Stations conforming with streptococci guide standards FS90
1991	7780	6938 (89.2%)	6080 (78.1%)	5144 (66.1%)*
1992	8505	7623 (89.6%)	6695 (78.7%)	6783 (79.8%)
1993	8799	7836 (89.1%)	7008 (79.6%)	7606 (86.4%)
1994	9185	8261 (89.9%)	7388 (80.4%)	7925 (86.3%)

* One country (Greece) did not submit results for faecal streptococci in 1991. The degree of compliance for the other three countries combined would be 5144 stations out of a total of 6683 identified bathing areas, *i.e.* 77.0%.