



**MEDITERRANEAN ACTION PLAN
MED POL**

UNITED NATIONS ENVIRONMENT PROGRAMME



FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

**FINAL REPORTS ON RESEARCH PROJECTS DEALING WITH THE
EFFECTS OF POLLUTANTS ON MARINE ORGANISMS AND COMMUNITIES**

**RAPPORTS FINAUX SUR LES PROJETS DE RECHERCHE TRAITANT DES
EFFETS DES POLLUANTS SUR LES ORGANISMES ET COMMUNAUTES MARINS**

MAP Technical Reports Series No. 80

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This volume is the eightieth issue of the Mediterranean Action Plan Technical Reports Series.

This series contains selected reports resulting from the various activities performed within the framework of the components of the Mediterranean Action Plan: Pollution Monitoring and Research Programme (MED POL), Blue Plan, Priority Actions Programme, Specially Protected Areas and Regional Marine Pollution Emergency Response Centre for the Mediterranean.

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

Cette série comprend certains rapports élaborés au cours de diverses activités menées dans le cadre des composantes du Plan d'action pour la Méditerranée: Programme de surveillance continue et de recherche en matière de pollution (MED POL), Plan Bleu, Programme d'actions prioritaires, Aires spécialement protégées et Centre régional méditerranéen pour l'intervention d'urgence contre la pollution marine accidentelle.

PREFACE

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

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

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

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

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

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

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

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

- information required for the implementation of the Convention and the protocols;
- indicators and evaluation of the effectiveness of the pollution prevention measures taken under the Convention and the protocols;
- scientific information which may lead to eventual revisions and amendments of the relevant provisions of the Convention and the protocols and for the formulation of additional protocols;
- information which could be used in formulating environmentally sound national, bilateral and multilateral management decisions essential for the continuous socio-economic development of the Mediterranean region on a sustainable basis;
- periodic assessment of the state of pollution of the Mediterranean Sea.

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

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

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

- development of reporting formats required according to the Dumping, Emergency and Land-Based Sources Protocols (activity B);
- formulation of the scientific rationale for the environmental quality criteria to be used in the development of emission standards, standards of use or guidelines for substances listed in annexes I and II of the Land-Based Sources Protocol in accordance with Articles 5, 6 and 7 of that Protocol (activity C);
- epidemiological studies related to the confirmation (or eventual revision) of the proposed environmental quality criteria (standards of use) for bathing waters, shellfish-growing waters and edible marine organisms (activity D);
- development of proposals for guidelines and criteria governing the application of the Land-Based Sources Protocol, as requested in Article 7 of that Protocol (activity E);
- research on oceanographic processes, with particular emphasis on surface circulation and vertical transport. Needed for the understanding of the distribution of pollutants through the Mediterranean and for the development of contingency plans for cases of emergency (activity F);
- research on the toxicity, persistence, bioaccumulation, carcinogenicity and mutagenicity of selected substances listed in annexes of the Land-Based Sources Protocol and the Dumping Protocol (activity G);
- research on eutrophication and concomitant plankton blooms. Needed to assess the feasibility of alleviating the consequences and damage from such recurring blooms (activity H);
- study of ecosystem modifications in areas influenced by pollutants, and in areas where ecosystem modifications are caused by large-scale coastal or inland engineering activity (activity I);
- effects of thermal discharges on marine and coastal ecosystems, including the study of associated effects (activity J);
- biogeochemical cycle of specific pollutants, particularly those relevant to human health (mercury, lead, survival of pathogens in the Mediterranean Sea, etc.) (activity K);
- study of pollutant-transfer processes (i) at river/sea and air/sea interface, (ii) by sedimentation and (iii) through the straits linking the Mediterranean with other seas (activity L);

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

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

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

Research area I - Characterization and measurement

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

Research area II - Transport and dispersion

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

Research area III - Effects

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

Research area IV - Fates/Environmental transformation

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

Research area V - Prevention and control

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

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

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

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

The present volume includes final reports on research projects dealing with the effects of pollutants on marine organisms and communities. Final editing and compilation of this volume was done by Mr. G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution) while Ms V. Papapanagiotou, FAO Secretary, was responsible for the typing.

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ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF MEDUSOZOAN
(MAINLY HYDROMEDUSAE) LIFE CYCLES

by

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ABSTRACT

A possible mechanism explaining the occurrence of blooms of seasonal planktonic organisms is proposed, suggesting that the presence of resting stages in their cycles could be a rather general feature and that the endogenous activation of such stages could set turns of biomass occupation in a given system. According to the model, biomass should remain constant in quantity but vary in quality, with different species alternating periods of abundance and rarity. The main problem for the verification of the model is the still incomplete knowledge of life cycle patterns of marine invertebrates. Several cycles of medusozoans are described, along with the environmentally induced modifications which could enhance the success of a given species in the competition for biomass occupation. It is concluded that blooms are a constant feature of planktonic communities, as suggested also by historical records, and that the problems they can cause to human activities depend only on the features of the species dominating over a certain period.

1. INTRODUCTION

The sudden occurrence of enormous swarms of gelatinous macrozooplankton (mainly Pelagia noctiluca) in the Mediterranean Sea over several years caused a sharp interest in such phenomena, with the promotion of long-term observations on the dynamics of blooms (UNEP, 1984; Rottini- Sandrini and Avian, 1986; UNEP 1991). Records scattered in the literature or in unpublished data reports have been collected demonstrating that such events are recurrent and probably not due to anomalies of environmental conditions. Boero (1991) elaborated a possible model for the processes generating blooms of seasonal animals. The model, here further generalized, proposed that the biomass present in a given environment is rather constant in quantity, but not in composition. The organisms actually composing the biomass of a system are present, in each year or multi-year cycle, in different proportions, even though the quantity of living matter tends to remain constant. This is rather evident from investigations on fishery activities (e.g. Balestra et al., 1976) but is still less well deducible from studies on non commercial animals.

According to the law of competitive exclusion, different species with similar niches cannot coexist if some dimension of the niche (e.g. food availability) is limited. This should result in the elimination of 'weak' species by 'stronger' ones, with a sharp decrease of diversity. Connell (1978) proposed a model which explained the maintenance of diversity in terms of a

certain (intermediate) level of disturbance which prevented the complete overcoming of 'weak' species by 'stronger' ones. Connell's intermediate disturbance hypothesis was proposed for rain forests and coral reefs; such environments are characterized by long-living species. Plankton is characterized by seasonal species which, especially in temperate areas, tend to be present just in a limited period of the year. Roughgarden *et al.* (1988) proposed another model, termed supply-side ecology, in which they explained the features of a given population of adults in terms of the history of the larvae from which the adults derive. This model is more explanatory for events such as blooms of seasonal organisms, as it happens in the plankton. In this case, in fact, recruitment is essential for the success of a given species in a given season.

The model proposed by Boero (1991) took also rare species into consideration, envisaging them as reservoirs of diversity for future scenarios. Dominant species should rarely suppress their competitors but, instead, should confine them in a status of low abundance. In a seasonal environment such as the planktonic one, a rare species, however, could become suddenly abundant due to the onset of favourable situations which allow it to outcompete formerly dominant species. Our perception of the composition of animal populations is biased by the period of observation and the accuracy of former studies. In the course of our lives we get used to certain situations and perceive them as 'normal', viewing every change as a catastrophe. Careful historical studies, however, often prove that the 'catastrophe' occurred already several times in the past. During these anomalous (as we perceive them) situations, often a rare species becomes suddenly abundant, outnumbering formerly common species. This is almost the rule in long-term studies of fisheries. The biomass of the system, however, remains quite constant over time, season by season. This does not mean, however, that every gross compartment of the marine environment (i.e. plankton, benthos, nekton) should constantly host a definite biomass. A plankton bloom of relatively big medusae such as *Pelagia*, for instance, could draw biomass from both zooplankton and nekton and, at the death of the different medusan cohorts, transfer it to the benthos. The degradation of such biomass, and its inclusion in benthic organisms, could require a rather long period, during which the building of a big planktonic (and nektonic) population could be limited.

Diversity, thus, tends to be low if measured as dominance of one or few species over the other ones. But should be always high, in terms of absolute number of species, if our samples could be efficient enough to comprise also the rarest organisms. Rare species should be just in a 'waiting list' for their turn for the occupation of the available biomass. In seasonal environments turns, as proposed by Roughgarden *et al.* (1988), should be decided by the outcome of recruitment. In his model Boero stated that seasonal organisms tend more and more to be discovered to have cycles with resting stages. They can be at the stage of eggs, fragments, not growing juveniles, and so on. Such stages have been found for several representatives of the planktonic taxa and the discovery of cycles of marine plankters with resting stages is flooding the recent literature (Boero, 1990), showing that what was considered as an exception (the presence of a resting stage in marine planktonic animals) is turning out to be a rather general rule.

The activation of resting stages to explain the sudden appearance of freshwater organisms is a well known and accepted phenomenon, and it is highly probable that the same could be true also in the marine environment, at least for some categories of organisms.

The model of Boero for plankton dynamics envisages a situation in which resting stages are activated by endogenous circannual clocks independent of major external cues. Such clocks have been demonstrated for some cnidarians (Brock, 1975). The onset of the favourable season, thus, should promote the growth of the populations of those organisms whose resting stages have a circannual clock which starts the active phase in the right moment. The organisms who start to be active earlier find unfavourable physico-chemical conditions, those active later are outcompeted by the species who have already built functional populations. Such events are rather random and allow a certain alternation in the occupation of the available biomass by seasonal organisms. The model applies also to phytoplankton, where resting stages are a well known fact and seasonal blooms are the rule. The presence of copepod eggs or algal cysts in the bottom muds is turning out to be a good tool to predict future blooms (e.g. Grice and Marcus, 1981; Imai, 1989; Lindley, 1990). The classic study of Werner (1963) for the hydromedusa Margelopsis haeckeli showed that such phenomena occur also in cnidarian life cycles.

Rare species, those usually not appreciated in general surveys and ecological studies, could remain latent with few functional individuals each year producing, however, a sufficiently large amount of resting stages to warrant a bloom when a favourable situation occurs.

Most of this scenario is purely hypothetical. Our knowledge of life cycles is still rather primitive, and we tend to generalize the few known cycles as rules for the groups to which the studied species are referred. This is obviously not correct, as the formerly unsuspected widespread presence of resting stages in planktonic organisms demonstrates.

Theory, thus, has to be followed by practice and, at present, it seems wise to concentrate our effort on the elucidation of the diversity of life cycles and investigate their ecological and evolutionary relevance.

Part of this work has been devoted to the search for blooms of gelatinous zooplankton in the Mediterranean, especially the Apulian coast (Ionian and Adriatic seas), and in the Bismarck Sea (Papua-New Guinea).

The main topic, however, was the rearing of medusozoans to link the polyp and the medusa stage in a complete cycle.

2. MATERIAL AND METHODS

Surveys have been carried out along the Apulian coast on a schedule ranging from monthly to bi-weekly. Standard stretches of beach were inspected after suitable wind conditions to look for stranded plankters. Other observations were regularly made in a site of the Ionian Coast (Porto Cesareo) by SCUBA diving. Fishermen were periodically interviewed to obtain reports about evidence of massive presence of planktonic animals, especially if gelatinous.

Life cycles of hydrozoans, the most abundant medusozoans, have been studied in the Mediterranean and in the Bismarck Sea (Papua-New Guinea). Polyps have been kept in little aquaria until medusa liberation, young medusae have been fed with *Artemia nauplii* until attainment of sexual maturity. Medusae collected in the plankton spawned in the laboratory and

the development of polyps from planulae has been observed. For suitable species such observations have been made in different seasons to find possible modifications of life cycle patterns according to seasonal conditions.

3. RESULTS

3.1 Surveys

3.1.1 The Mediterranean

No relevant bloom of conspicuous gelatinous zooplankton possibly harmful to human activities has been recorded in the investigated period (1986-1990).

In 1986 (Boero and Minelli, 1986) the cubozoan Carybdea marsupialis has been recorded for the first time from the Adriatic Sea. Its presence caused minor stings in a few swimmers. Cubozoans have been observed since a long time from the Adriatic, but their presence apparently was never officially recorded in scientific literature (Ghirardelli, personal communication).

In 1987 many specimens of the scyphozoans Cotylorhiza tuberculata and Rhizostoma pulmo have been observed in the Gulf of Naples, along the Ligurian Riviera and in the Ionian Sea. They never formed massive swarms.

In 1988 a massive stranding of the pleustonic hydroids Velella velella, accompanied by the less abundant Porpita porpita, occurred along the Ionian coast of Apulia. Such events have not been recorded since twenty years of continuous observations of local conditions carried out at the Stazione di Biologia Marina del Salento, at Porto Cesareo. Velella, formerly very abundant in the Mediterranean, became rare in the area in the last decades. It has been further recorded along the Ligurian coast in 1989 (Relini, 1990) and also in Spain (Garcia Rubies, personal communication). Together with Velella and Porpita were also big Aequorea leptomedusae, but in smaller numbers. Siphonophores were also present in the stranded plankton. Fishing nets of local fishermen were clogged by siphonophores.

In April 1990 a concentration of gelatinous zooplankton was observed during a SCUBA dive in the Ionian Sea. It was composed mainly of ctenophores, hydromedusae and siphonophores. The day after the observation the wind changed and the aggregation disappeared.

3.1.2 The Bismarck Sea

The same phenomena were searched for in the Bismarck Sea during June-August 1986, February-March 1987 and January-February 1989. The monitored area, centered at Laing Island (Madang Province), is completely immune to the effects of local human activities and could be considered as very near to completely 'natural' conditions. Gelatinous zooplankton, there, occurs in continuous swarms of alternating species, concentrated by Langmuir currents as described by Hamner and Schneider (1986). Such concentrations usually showed the dominance of one species, even though other species were present. Different zones, several metres apart from each other, could harbour a completely different medusan fauna. Long term observations

(Bouillon *et al.*, 1986) support the results obtained during the present studies. In 1986 a bloom of Pelagia noctiluca was observed, with millions of small specimens.

Scyphomedusae and cubozoans were only occasional, the greatest majority of gelatinous zooplankton being composed of hydromedusae, siphonophores and ctenophores.

These observations were carried out at the King Leopold III Marine Laboratory at Laing Island. Samples and observations in other coastal areas and islands revealed a lower abundance of gelatinous zooplankton.

3.2 Cnidarian life cycles

The scope of this part of the project was to summarize the main types of cycles known for hydrozoans and to ascertain if the presently known categories apply to all species. Two previously unknown or poorly understood types of cycle have been discovered and described.

3.2.1 Swimming gonophores

Boero and Bouillon (1989a) studied the occurrence of apparently anomalous medusa stages in thecate hydroids. According to current taxonomy thecate hydroids should produce leptomedusae, with gonads on radial canals, whereas athecate hydroids should produce anthomedusae, with gonads on the manubrium. This proves to be true in most cases, but more and more thecate hydroids are discovered to produce ephemeral medusae (currently termed medusoids), released with already ripe gonads, non-feeding, and living just a few hours, which could be classified as anthomedusae owing to the position of their gonads. Such medusoids, in fact, instead of having gonads on the radial canals, as the medusae of other thecate hydroids, bear gonads on the manubrium, the typical position for the medusae of athecate hydroids.

The comparison of the anatomical features shown by a gradual series of medusa suppression in Leptomedusae (Fig. 1) suggested a possible interpretation for this apparently anomalous type of morph in the cycle of some Leptomedusae. The following series, and the proposed examples, does not represent a linear sequence of modifications along a single evolutionary trend. It is derived from situations described in recent species which could show what could have been the steps leading to medusa reduction in leptomedusan life cycles.

Fig 1, I: the blastostyle (i.e. the reduced reproductive polyp) buds off medusae which are liberated. The manubrium is a projection of the blastostyle and is homologous with the spadix. The sex cells are borne on the radial canals (free medusae).

Fig. 1, II: The blastostyle buds off medusoids, with a reduced or even absent manubrium, which are liberated with already ripe sex cells on the radial canals (free eumedusoids).

Fig. 1, III: The blastostyle buds off still further reduced medusoids, which remain in the gonotheca, with gametes on the rudiments of the radial canals (fixed eumedusoids).

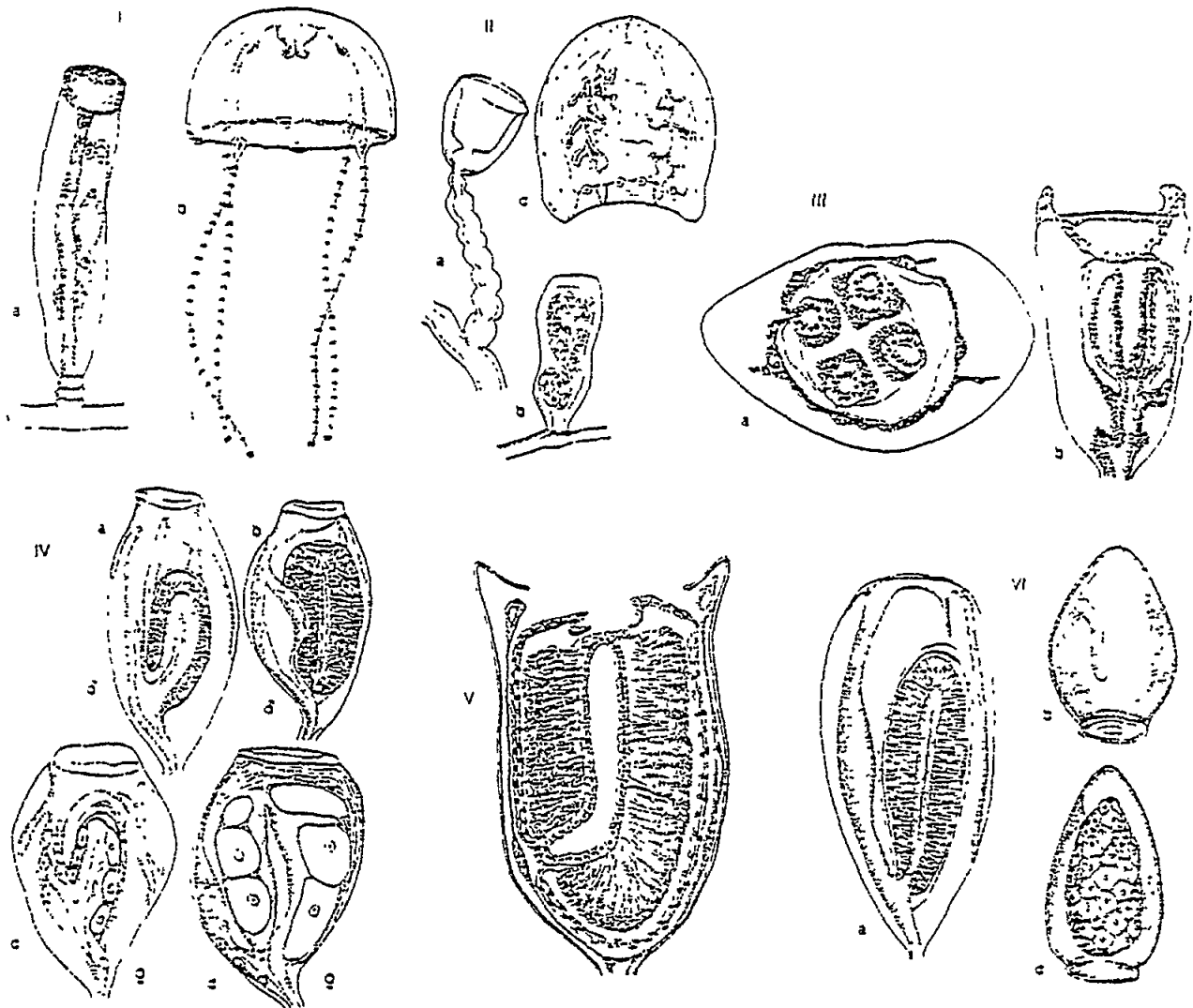


Fig. 1 Possible steps leading from free medusae to swimming gonophores. I, *Clytia linearis*, a: gonophore with blastostyle budding medusae, b: newly liberated medusa. II, *Orthopyxis integra*, a: polyp and b: gonotheca with forming eumedusoids, c: liberated eumedusoid; III, *Campanularia hincksii*, a: transverse section of gonotheca, with fixed eumedusoid, b: longitudinal section of gonotheca showing gametes on the radial canals of the fixed medusoid; IV, *Dynamena pumila*, a: immature and b: mature male gonotheca with gametes on the spadix, c: immature and d: mature female gonotheca with gametes on the spadix. V, *Plumularia obliqua*, longitudinal section of gonotheca with gonophore bearing gametes on the spadix, showing rudimentary medusan structures. VI, *Amphisbetia operculata*, a: longitudinal section of a gonotheca bearing gametes on the spadix, b, c: liberated swimming gonophores. (After Boero and Bouillon, 1989a)

Fig. 1, IV-V: The blastostyle buds off a spadix which is surrounded by the sex cells. Some rudiments of medusoid structures remain in the fixed gonophore (cryptomedusoids, heteromedusoids).

Fig. 1, VI: The gonophore having the sexual elements around the spadix (as in case IV) and functional medusoid structures is secondarily liberated as a new morph, termed swimming gonophore.

With this reconstruction it is proposed that the medusa stage has been gradually suppressed, but that traces of its genetic codification remained in the species with fixed gonophores. The anomalous 'medusae', with gonads on 'manubrium', of some thecate hydroids are here interpreted as a mosaic of medusan and fixed gonophore features, and are proposed as a new stage in the life cycle of cnidarians. Boero and Sarà (1987), and Boero and Bouillon (1987) called for such a pedomorphic mechanism to explain the presence of apparently anomalous morphs in the cycles of hydromedusae (Fig. 2).

3.2.2 Solitary gonotheca with no hydroid development

Laodicea indica is a common species at Laing Island (Bismarck Sea), where it forms extensive swarms in the dry season being, nevertheless, present also in the wet season. Bouillon et al. (1991) followed the cycle of this species in both seasons, rearing planulae deriving from eggs fertilized in the laboratory. The development proved to be consistently different in the two seasons (Fig. 3).

- Wet season. The planula settles, gives rise to a normal hydroid colony, with the features of the genus. Eventually, after colony growth, medusae develop within gonothecae and are liberated. Each planula, thus, gives rise to a high number of medusae which are born long after planula settlement.
- Dry season. The planula settles as above but, instead of producing a hydroid colony, immediately produces a gonotheca with a single or rarely two medusa buds. Medusa liberation occurs a few hours after settlement. In this case one planula almost immediately gives rise to one (or two) medusae.

This kind of cycle was previously unknown and shows how the same species can modify its life history according to environmental cues. The dry season, when swarms occur, apparently allows for fast growth of the medusan population and the hydroid (representing a functional and temporal gap between two medusan generations) is abolished from the cycle, with the onset of a 'one planula-one medusa' situation. The wet season, on the contrary, should be less favourable and the hydroid becomes a functional morph which has access to different food sources than the medusa, thus building the future generation of the adult stage. In this case the situation is 'one planula-a hydroid colony-many medusae'.

3.2.3 Previously unknown hydromedusan cycles

The cases described above could be considered rather exceptional if viewed in the framework of the already known diversity of hydromedusan life cycles. Most hydromedusan species, however, have unknown cycles and it

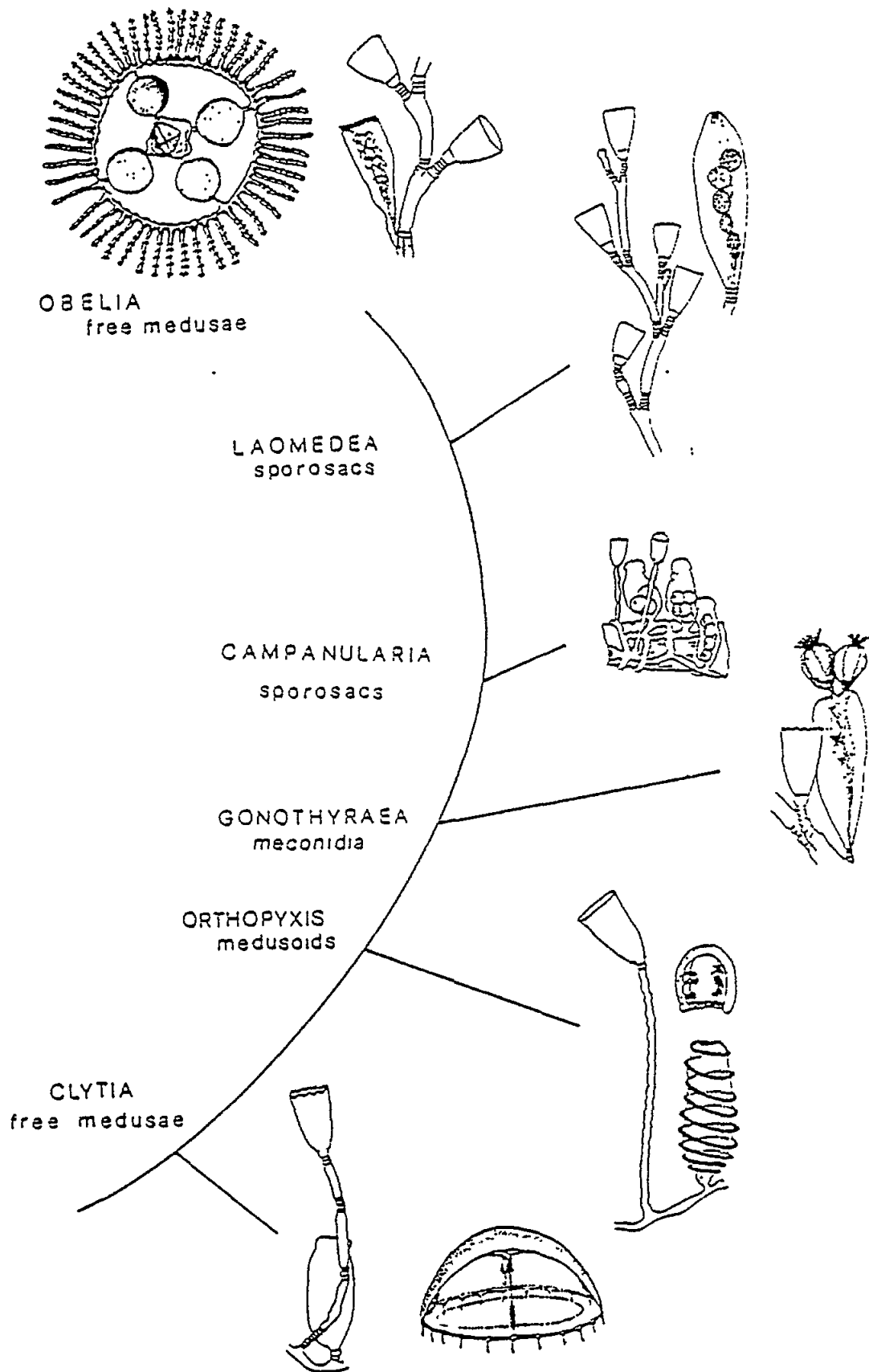


Fig. 2

A possible phylogeny of the Campanulariidae, based on the gradual reduction of their motile sexual stages, explaining the presence of the highly modified medusa of *Obelia* as a case of medusa re-expression after medusa suppression. (After Boero and Sarà, 1987)

could be possible that the known ones are the easier to study and that many new types will be discovered in the future. The basic objective of the present research was, then, to increase the number of species with a known cycle. Such studies are usually of no immediate ecological interest, but constitute a data base which will prove useful in case of sudden explosion of these species. A detailed account of these cycles is outside the scope of the present paper, so just some basic information and figures will be given.

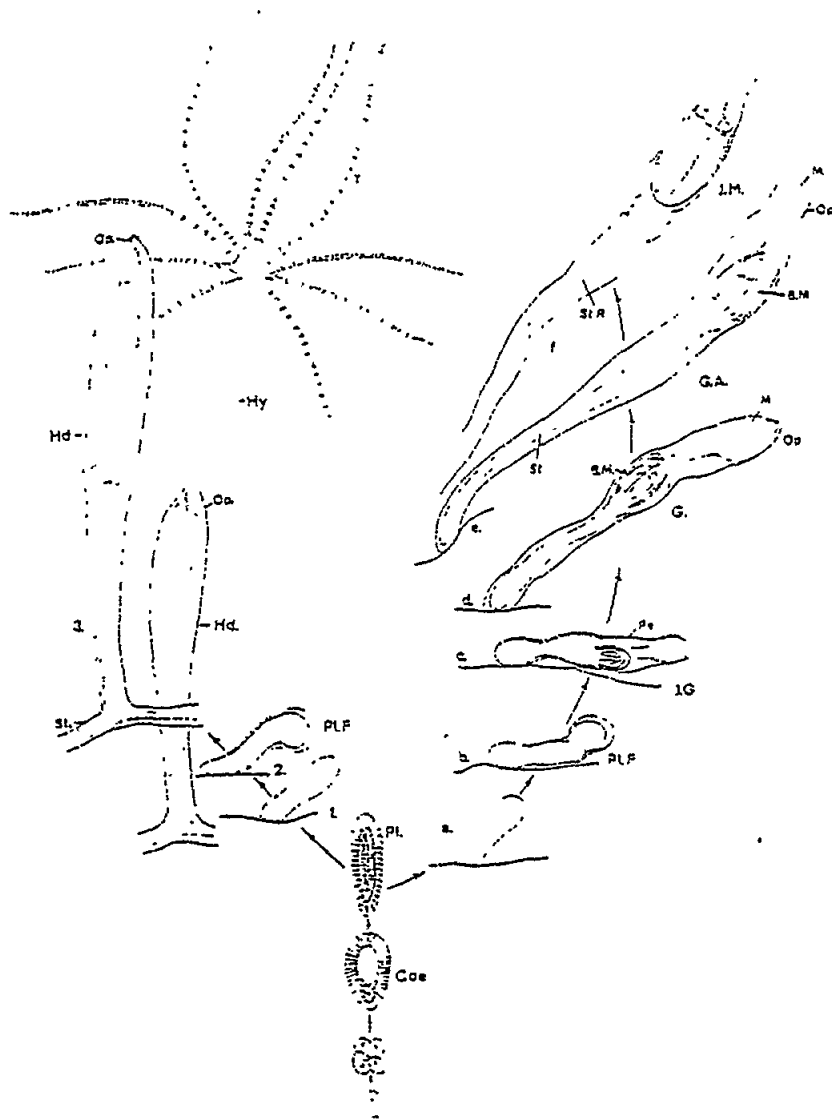


Fig. 3 *Laodicea indica*. Diagram of life cycles in wet season (left 1-3) and dry season (right: a-f). B.M.: medusa bud; Coe: gastrulating coeloblastula; G: gonophore; G.A.: adult gonophore; Hd: hydrotheca; Hy: hydranth; J.G.: young gonophore; J.M.: young medusa ready to be liberated; M: terminal plate; Op.: Operculum; Pe.: perisarc; Pl: planula; PlF.: settling planula; St.: stolon; St.R.: degenerating stolon; T.: tentacle. (After Bouillon et al., 1991)

Octotiar russelli

This species was known from the medusa stage only. Boero and Bouillon (1989b) discovered that its hydroid is symbiotic with bryozoans and succeeded in rearing the medusa from newly-liberated to adult stage (see Figures 4a, 4b and 4c).

Stomotoca atra

Also this species had an unknown cycle and was based just on the medusan generation. Boero and Bouillon (1989b) described its cycle (Figs. 5a and 5b) and showed that the hydroid is different from the parasitic one described by Larson (1982) for Stomotoca pterophylla.

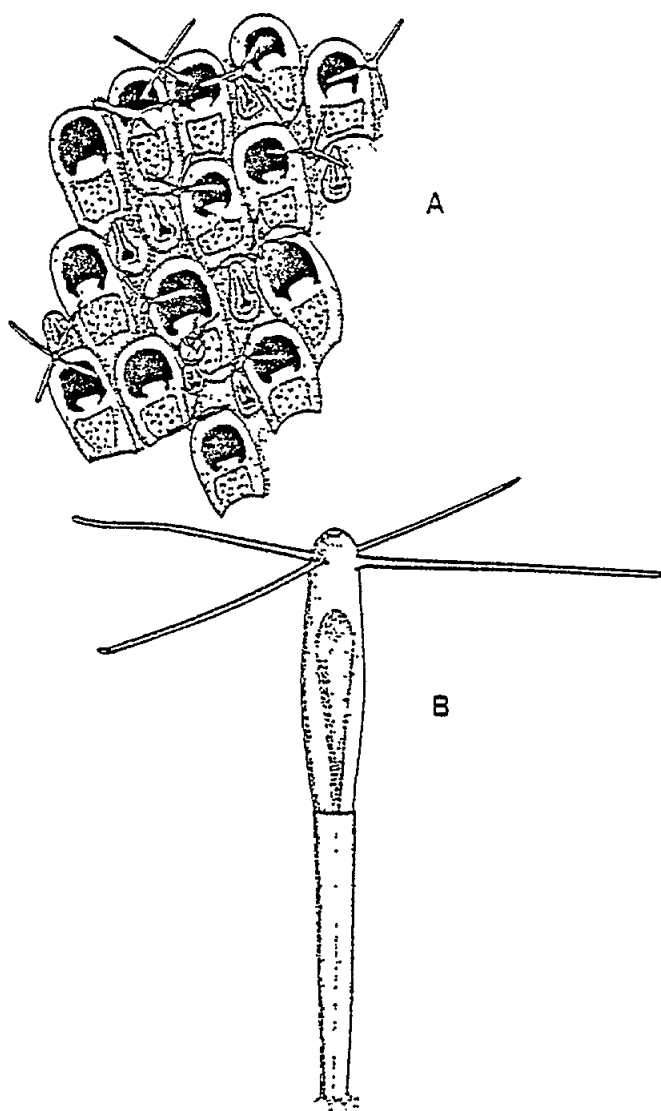


Fig. 4a Octotiar russelli. A: colony with medusa bud, growing on a bryozoan; B: hydranth. (After Boero and Bouillon, 1989b)

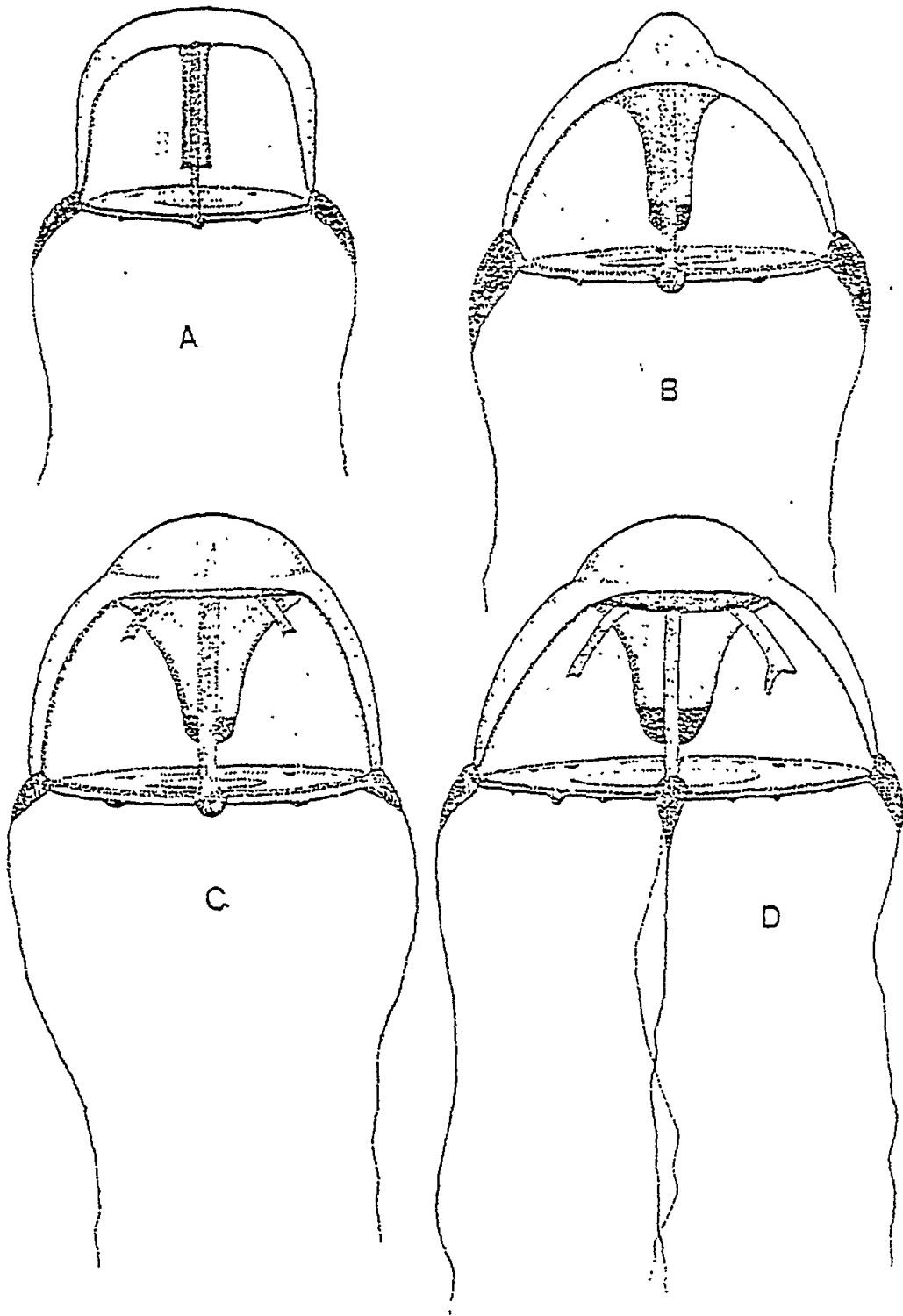


Fig. 4b Octotiarra russelli. A: newly released medusa; B: five-day-old medusa; C: nine-day-old medusa; D: fifteen-day-old medusa. (After Boero and Bouillon, 1989b)

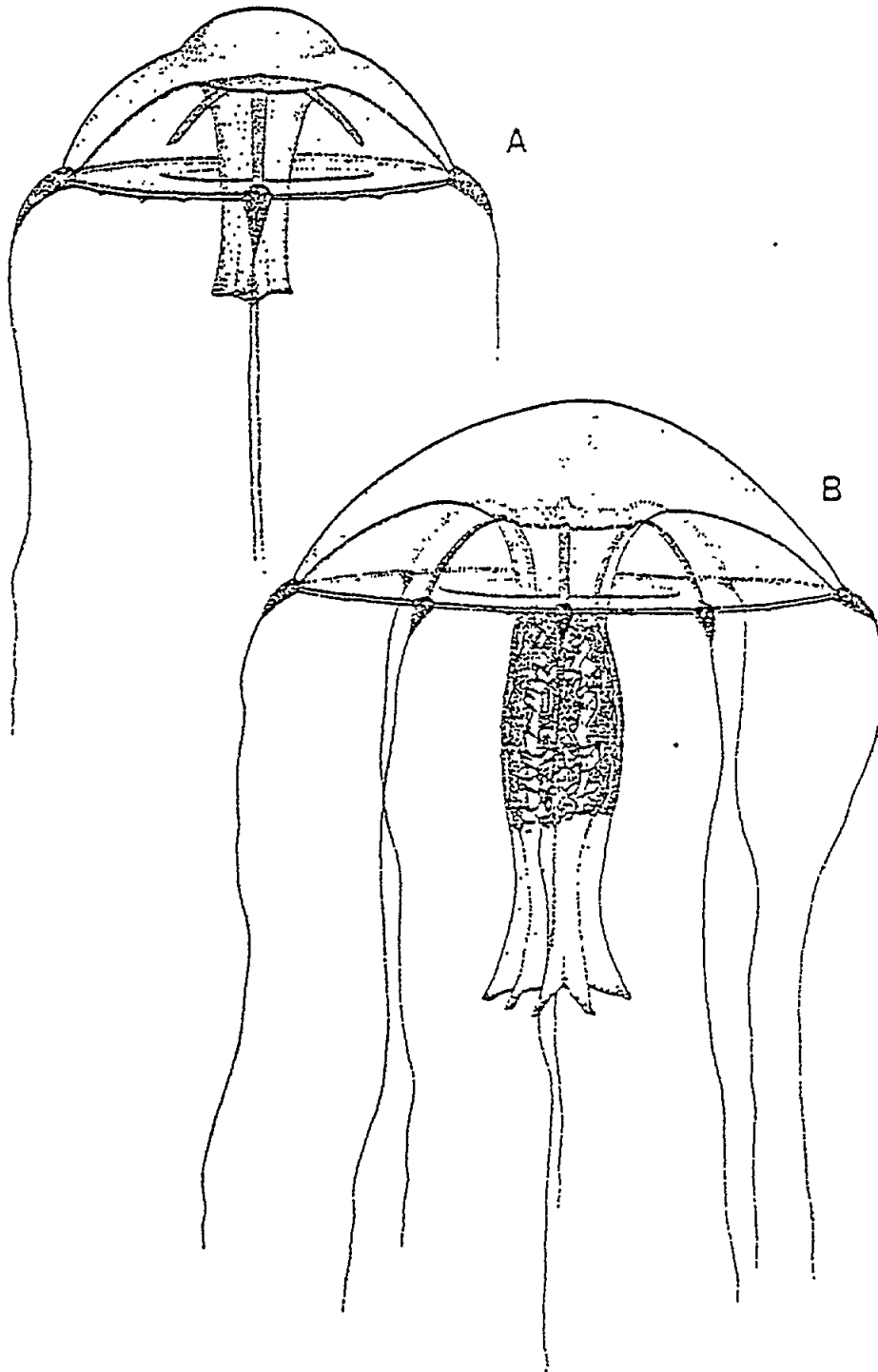


Fig. 4c A: Twenty-day-old medusa; B: adult medusa collected from plankton (After Boero and Bouillon, 1989b)

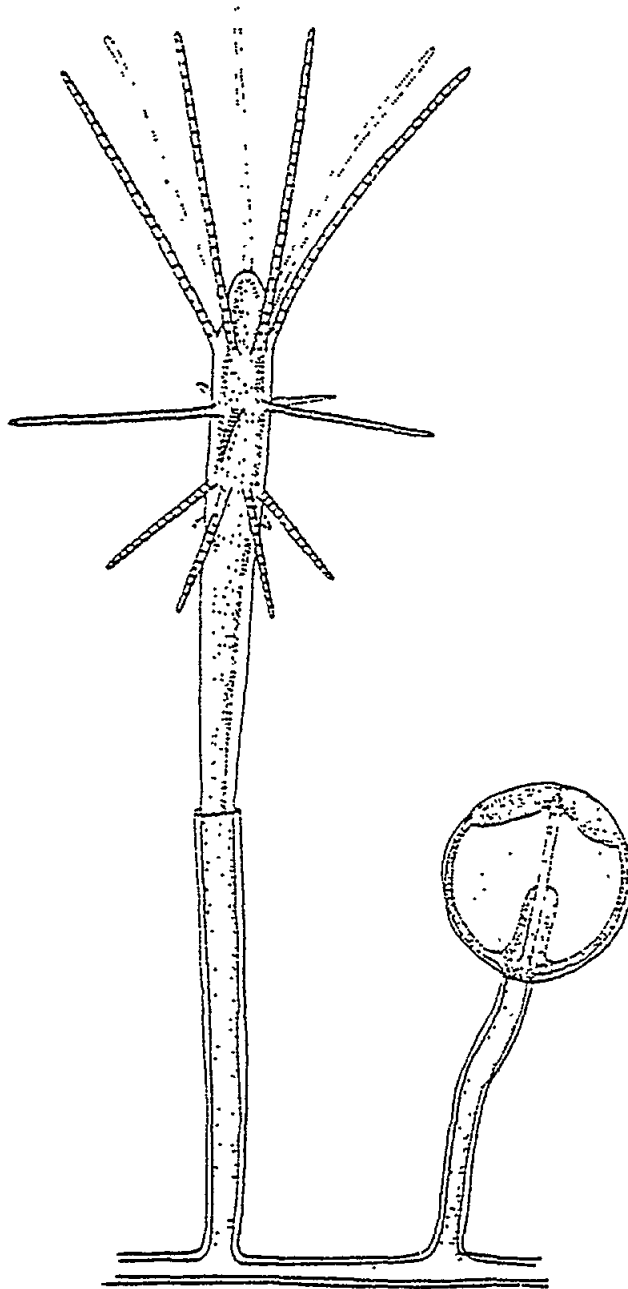


Fig. 5a Stomotoca atra. Hydroid and medusa bud (After Boero and Bouillon, 1989b)

Hydrichthys mirus

This species was known from the hydroid stage only, the polyps being parasitic on fish just as those described by Larson (1982) for Stomotoca pterophylla. The finding of a fish parasitized by the hydroid, allowed to obtain several medusae which were reared to maturity (see Figures 6a, 6b and 6c) (Boero et al., in press). They proved different from those of Stomotoca pterophylla in spite of the high resemblance of the hydroid stages. The taxonomic and evolutionary relevance of this is discussed in detail by Boero et al. (1991).

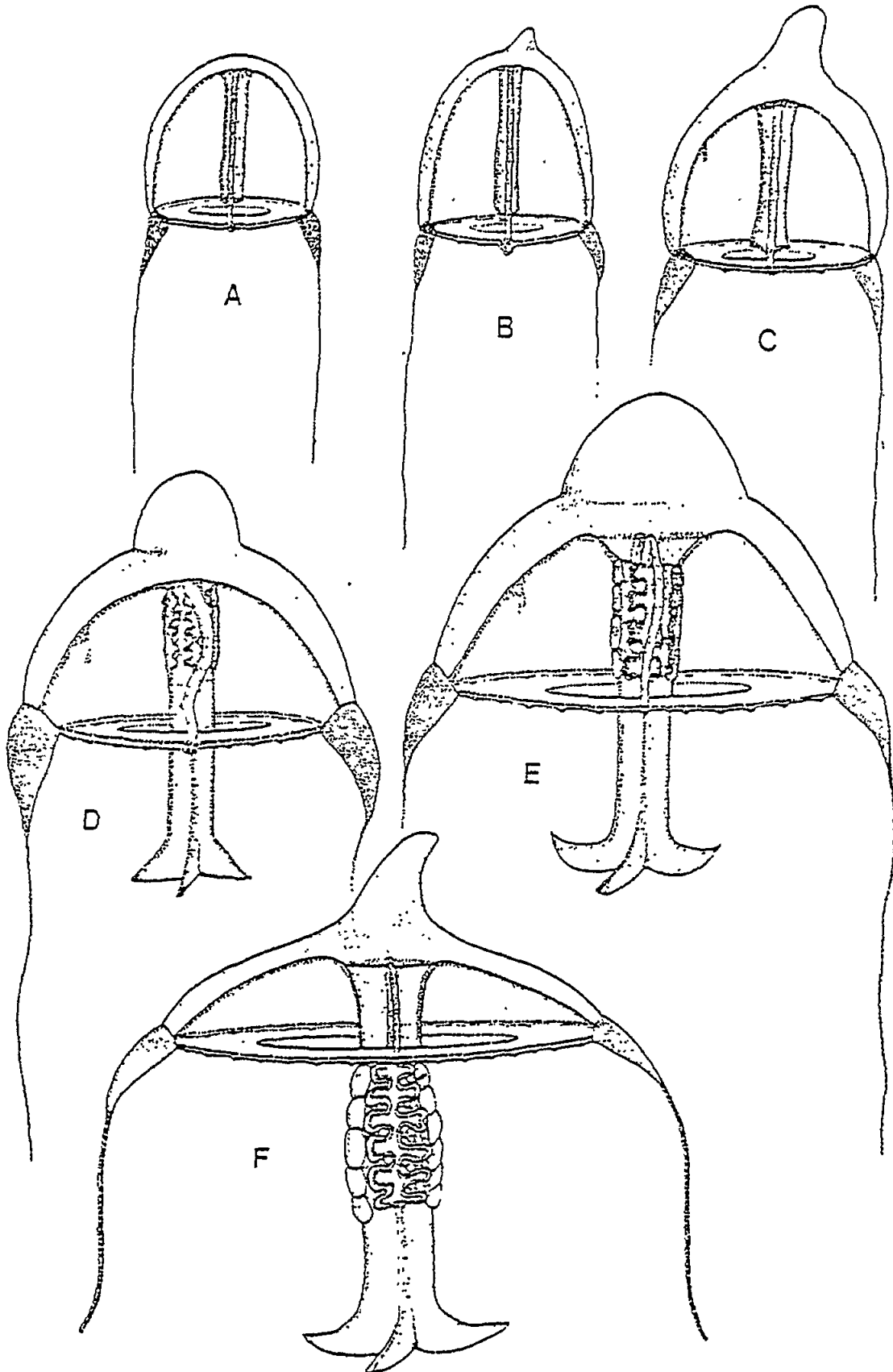


Fig. 5b A: newly released medusa; B: two-day-old medusa; C: eight-day-old medusa; D: twelve-day-old medusa; E: twenty-day-old medusa; F: adult medusa collected in the plankton (after Boero and Bouillon, 1989b)

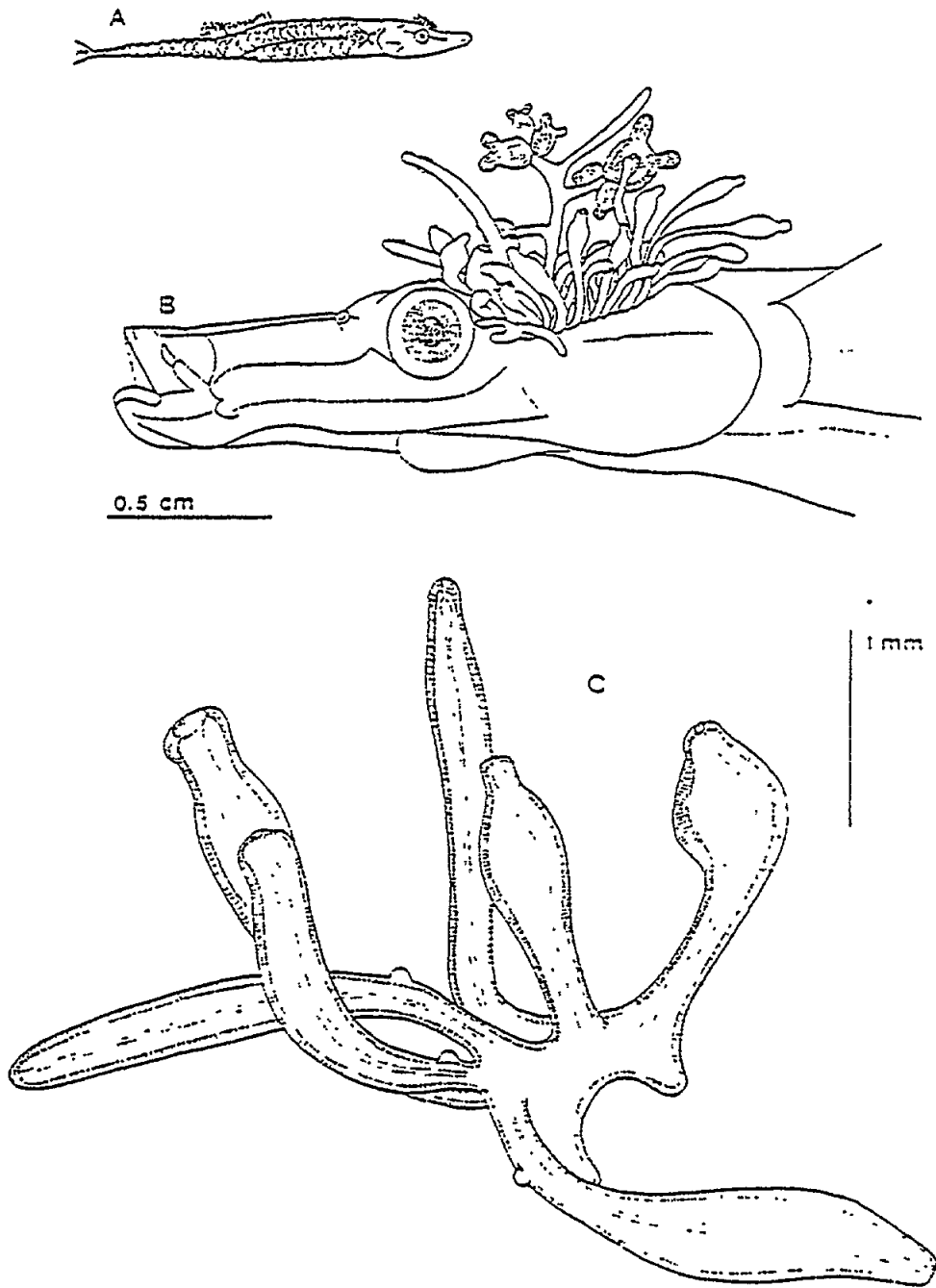


Fig. 6a Hydrichthys mirus. A: syngnatid fish parasitized by two hydroid colonies; B: detail of fish head with general view of hydroid colony; C: colony detached after the death of the host fish (after Boero et al., 1991)

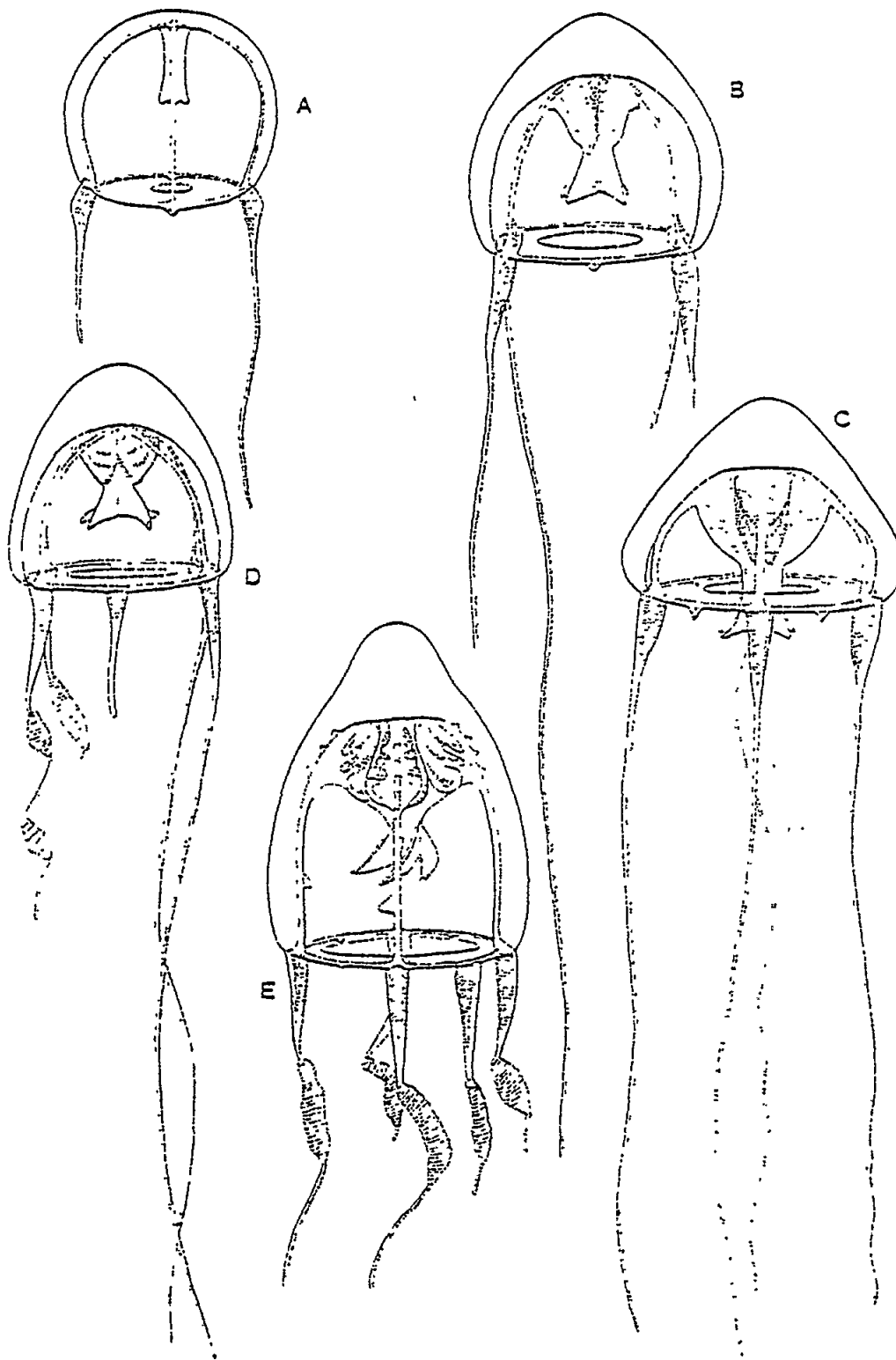


Fig. 6b A: newly released medusa; B: four-day-old medusa; C; same as in B, but after feeding; D: six-day old medusa; E: ten-day-old medusa (After Boero et al., 1991)

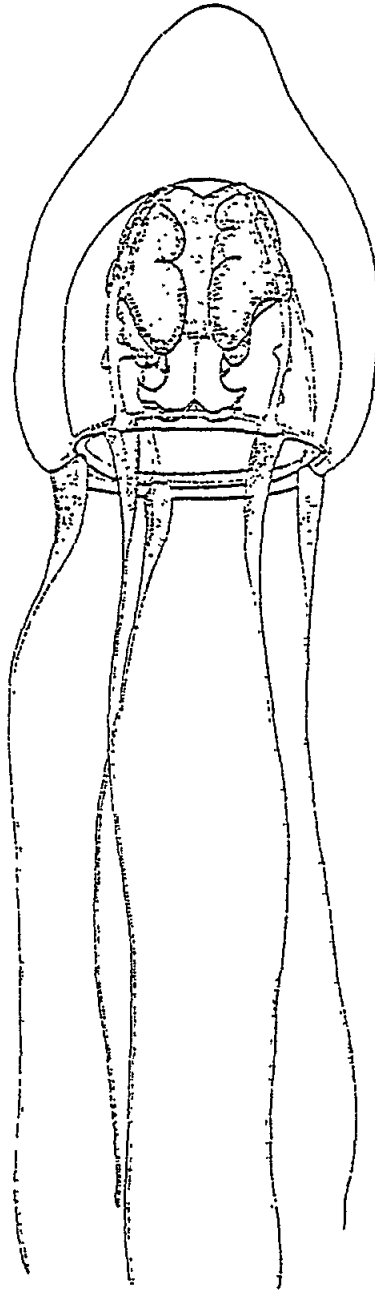


Fig. 6c Adult medusa, fifteen-day-old (After Boero et al., 1991)

Clytia linearis

This species is common both in the Bismarck Sea and in the Mediterranean. Its cycle has been studied in both seas (Boero and Sarà, 1987; Boero and Bouillon, unpublished observations) and some differences in the dynamics of the life history have been found. In July-August in the Mediterranean, and in both the wet and the dry season in the Bismarck Sea, the hydroid liberates immature medusae which grow and attain maturity in two-three

weeks, with increase in size, number of tentacles and statocysts. In September-October in the Mediterranean, however, the newly liberated medusae have already ripe gonads, live a few days, spawn and die. This contracted cycle is not followed by an increase in the occurrence of the benthic hydroid which, on the contrary, could not be found in the field. It is probable that the planulae settle and encyst in form of resting hydrorhizae, ready to give rise to new colonies at the onset of the following warm season.

The species has a stable life cycle in tropical conditions, whereas in the temperate climate of the Mediterranean is active only in the warmer part of the year, being able to shift from normal medusae to short-lived medusae, to resting stages at the approaching of the adverse season.

Life cycles of Zancleoidea

The superfamily Zancleoidea comprises several genera with poorly known cycles. Research carried out at Laing Island (Bismarck Sea), Porto Cesareo (Mediterranean Sea), Bodega Bay (Central California) allowed the study of several cycles, with discovery of new species and genera. The results are still in course of elaboration and a detailed account is premature. The superfamily, however, shows a series of species with hydroids symbiotic with bryozoans and reduced medusae (referable to an undescribed genus and to Halocoryne), species with normal medusae and reduced polyps symbiotic with bryozoans (Zanclea and one or possibly two new genera). These findings seem to reconcile with the hypothesis outlined by Boero and Sarà (1987) and Boero and Bouillon (1987) according to which, through paedomorphosis, the morphs of a cycle can vary in shape and ecology independently from each other, so to have evolutionary trends with conservative medusae and innovative polyps or vice-versa.

Hebella

Boero (1980) described the previously unknown medusa of Hebella parasitica showing that its morphology could be referable to that of anthomedusae rather than that of leptomedusae, in spite of the clear thecate (and thus leptomedusan) features showed by the hydroid. The solution of this apparent incongruence in the separate hydroidan and medusan classifications has been proposed by Boero and Bouillon (1989a) with the introduction of the concept of 'swimming gonophore', so that the motile sexual stage of Hebella parasitica is here interpreted as a swimming gonophore. The life cycles of other species of Hebella, however, remained unknown, and just some species are reported to produce medusae known from the gonotheca or as newly liberated immature individuals. It has been possible to obtain Hebella medusae from colonies reared at Laing island. The medusae were extremely delicate and could not reach maturity in the laboratory. It has been possible, however, to ascertain that their morphology is typical of Leptomedusae. The two studied species had new born medusae with ocelli, and one showed doubling of radial canal number. These findings could justify referral of Hebella parasitica to a separate genus, but are still too incomplete to reveal if the typical medusa of other Hebella species could be referred to an already known medusan genus. A revision of the genus is being elaborated (Boero et al., in preparation).

3.3 Zoogeography and life cycle patterns of Mediterranean hydromedusae

This subject has been dealt with in detail by Boero and Bouillon (1993) who described all known types of life cycle of hydromedusae (Fig. 7), referred the 346 hydromedusae known to live in the Mediterranean to a type of cycle, and divided the species into contingents of different zoogeographical affinity. The obtained results are somewhat surprising in that the distribution of the species seems not to be influenced by the presence of a medusa in the cycle.

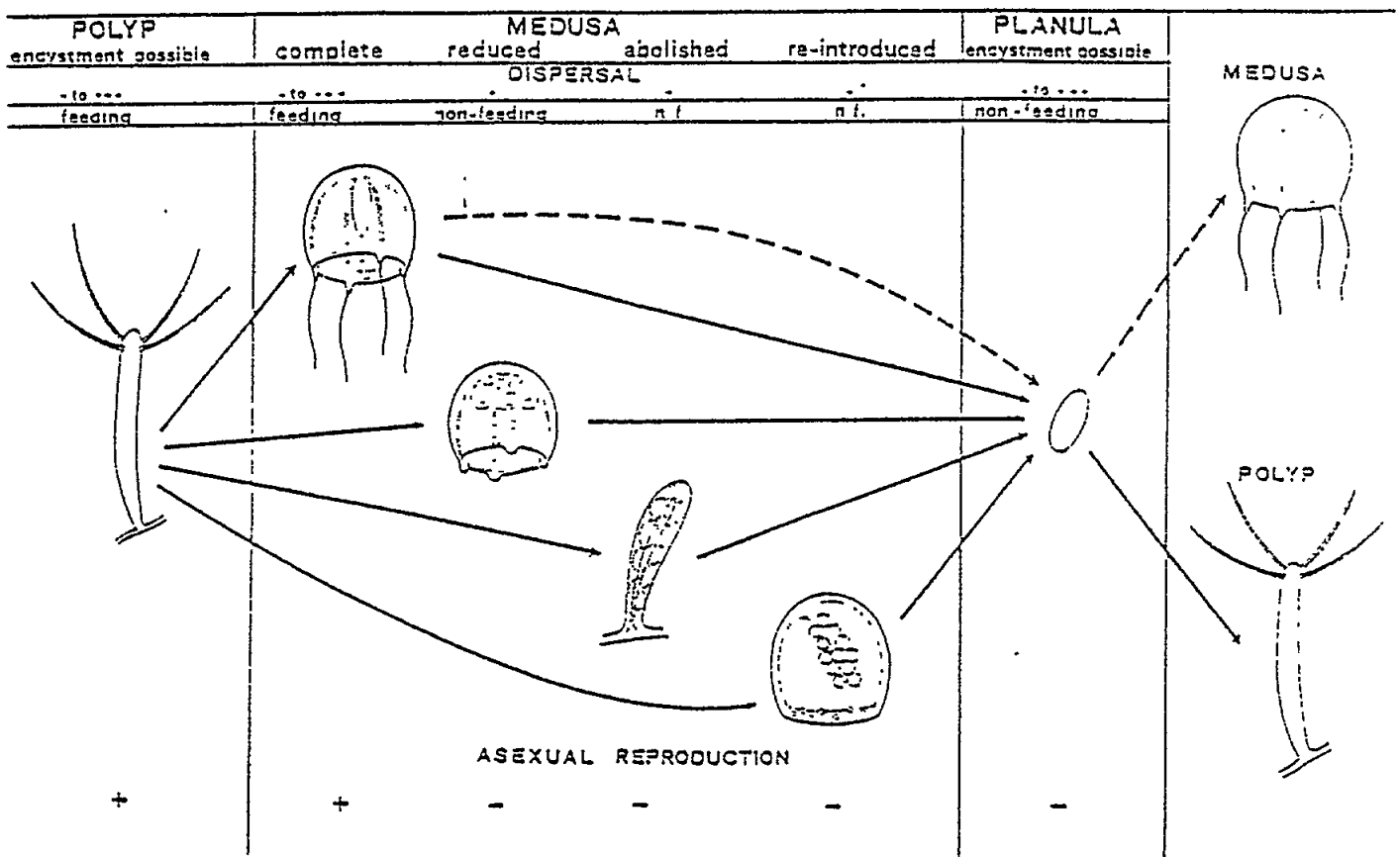


Fig. 7 Life cycle patterns of hydromedusae, with dispersal possibilities (from - to +++), presence (+) or absence (-) of asexual reproduction, and trophic value of the various stages. Broken arrows: direct development, with no hydroid stage; solid arrows: indirect development with hydroid stage

It is proposed that dispersal is obtained also through the polyp and the planula stage, due to the possibility of being transported by floating objects. The presence of a medusa makes dispersal efficient over short periods of time. In fact, among the few Indo-Pacific species of the Mediterranean hydromedusan fauna, those with medusa prevail over those with the hydroid stage only. Such species are possibly Lessepsian migrants and are mainly recorded from the Eastern Mediterranean. Since Lessepsian migration is

possible since 1869, when the Suez Canal was opened, only the species with a more efficient dispersal could spread to the Mediterranean in such a short period. The majority of the Mediterranean hydromedusan fauna, however, has a clear Atlantic affinity and entered the basin from the Strait of Gibraltar, after the Messinian crisis (between 6 and 5 My BP). Such a long period allowed the entrance and the establishment of species with a vast array of life cycle patterns which, in the long term, proved to be rather unimportant in the dispersal possibilities of the species. Half of the 67 endemic species, for instance, have a medusa stage in their cycle, whereas it should have been expected that the species with a suppressed medusa stage should have contributed more to the endemic contingent than those with medusae. The same is valid for the contingents with wide distribution. Contrary to what was expected, the species with a suppressed medusa stage almost equalled those with medusae in the circumtropical and the cosmopolitan contingents.

The Mediterranean hydromedusan fauna is rich and rather original, owing to the high proportion (19.5 %) of endemic species, but has been, and probably still is, dependent on a supply of species which entered from Gibraltar (and now from the Suez Canal). The sharp surface temperature interval between the warm season (25-28NC) and the cold season (10-13NC) allow the coexistence of seasonal medusozoan faunas of different affinity.

4. DISCUSSION

4.1 Surveys

The analysis of different situations, such as that of the Mediterranean (a temperate sea) and that of the Bismarck Sea (a tropical sea) showed that in both cases blooms of gelatinous zooplankton are quite common phenomena. At Laing Island (Bismarck Sea), furthermore, the abundance of medusae is probably exceptional even for that area and is probably due to particular hydrographic conditions which tend to concentrate the animals.

Both the Mediterranean and the Bismarck Sea show different composition of gelatinous zooplankton in different years, seasons, and days.

Daily differences are mainly due to winds causing currents which can bring planktonic animals towards the coast or carry them away. This is mainly true during calm weather conditions, when regular breezes with alternate direction occur. The local composition of gelatinous plankton has been seen to vary within hours.

Seasonal differences are due to the features of the biological cycle of the species involved. Nearly all planktonic species are seasonal and so adapted to take advantage of conditions occurring over restricted periods. Each species has its typical period of occurrence, usually linked to a particular season. Such periods may be different for populations of the same species, living at different latitudes. The same species, furthermore, could vary its life history in the different seasons, as it happens for Laodicea indica and Clytia linearis.

Yearly differences are probably due to competition among species for the utilization of available resources, as proposed by the model hypothesized by Boero (1991) and summarized in the introduction.

The alternation in the occupation of the biomass of gelatinous species is not so evident when conspicuous species such as Pelagia noctiluca are substituted by less evident forms such as hydromedusae, siphonophores or ctenophores. The blooms of Velella and siphonophores identified during the first years covered by the present investigations had local effects similar to those recorded for Pelagia. They have been less noticed because they occurred in spring, when the coasts are still not widely populated by tourists.

Conventional sampling by plankton nets, furthermore, tends to be inefficient for animals such as ctenophores, which are readily destroyed by touch. It is possible that many gelatinous species not living directly at the surface could pass unnoticed if not searched for by direct observation by SCUBA or submersible diving.

The re-appearance of Velella after a long period of absence of records from the Mediterranean further supports the idea of an alternation of the occupation of the biomass by different species in different periods. Also the recurring 'pulses' of Pelagia recorded in the last century could be explained by Boero's hypothesis of alternate biomass share.

Obviously not all gelatinous species are equally conspicuous and equally noticeable for their effects on human activities, or have the same depth preferences. The study of gelatinous macrozooplankton should be carried out by direct observation at different depths, using SCUBA and submersible dives over a regular schedule. Only a co-ordinated protocol of investigations with proper methodology and over a sufficient period and area could allow confirmation or rejection of Boero's hypothesis which, at present however, seems to be supported by indirect evidence.

4.2 Life cycles and distribution

Two new types of cycles have been described, one with swimming gonophores, and one with almost complete reduction of the polyp stage. Other investigated species showed peculiar features of their life cycles, and it is more and more evident that the stage of knowledge about these topics is at present in a rather fluid state. Also the meaning of the medusa in the cycle is not so obviously linked to a higher dispersal potential, as demonstrated by the analysis of the zoogeography of the Mediterranean hydromedusan fauna.

General accounts must be continuously changed as soon as new types of cycle are described, and this calls for further detailed studies on life cycles. Our knowledge of this topic still requires effort in basic research, as the general framework still exhibits enormous gaps.

It is not unwise, at this point, to hypothesize the presence of a resting stage in the cycle of Pelagia even though no direct evidence supports this view.

5. CONCLUSION

The work carried out in these last years on the gelatinous zooplankton of the Mediterranean supports the view that the blooms of Pelagia have not been anomalous and that

they can be considered as events which periodically occur in the alternation of biomass occupation by the species living in a given system.

Having proposed a possible mechanism for such events, however, is not sufficient to allow prediction of their occurrence. The study of life cycles, life histories and larval biology could give information about the building up of a situation which could lead to a bloom. Such information however, should not be used to contrast, for instance, a previewed Pelagia bloom with chemicals or introduction of possible predators. We should, instead, learn to live in a dynamic environment in which changes are not always synonym of catastrophes. This optimistic view, however, should not be interpreted as an exactly opposite attitude. Changes must be investigated, and their relevance in a normally dynamic situation must be evaluated case by case.

The only possible way to understand the dynamics of our environment is to promote long-term observations in standard sites and by standard methods. The most convincing evidence that Pelagia was not a catastrophe has been that such events have been recorded many times in the past. Records, however, were so scattered to give almost no more information than the record itself. Recent cuts in research funding tend to abolish long term studies, judged as dull and little innovative. Needless to say, the story of Pelagia shows the enormous importance of such studies which, however, must be centered on further knowledge of the cycles of the species involved. The presence of benthic resting stages in animals formerly considered as holoplanktonic teaches us that plankton surveys in coastal waters are useless if not paralleled by benthic samplings devoted to the study of the resting stages. The very concept of holoplankton, at least in coastal waters, seems to be strongly challenged by recent findings. Pelagia has a holoplanktonic cycle in certain conditions, but we know that other cnidarians can change the features of their cycle according to the prevalent environmental conditions. This could apply to Pelagia too. Future blooms could be started by populations of 'seeds' now resting on the bottom of our seas.

6. ACKNOWLEDGEMENTS

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IDENTIFICATION OF BIOLOGICAL INDICATORS OF HEAVY METALS AT THE SITE OF A SMELTING FACTORY

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ABSTRACT

Seven heavy metals, Co, Cr, Cu, Fe, Mn, Ni and Zn were measured in marine sediments, plants and invertebrates in the vicinity of a ferro-nickel smelting plant and in a control site in Greece. The concentrations of metals in the sediment were higher than those found in the average unpolluted Greek coastal sediment. Animals collected near the smelter also had higher concentrations than those obtained from the control site. Higher levels were observed in the gastropod molluscs, where the concentrations in the viscera were statistically higher than those in the muscle. From the species examined, Cerithium vulgatum showed the highest concentrations of metals and could be used as a "sentinel species" for metal contamination.

1. INTRODUCTION

The small town of Larymna is situated on the eastern coast of mainland Greece in the North Evoikos Gulf (Fig. 1a). The town is on the north western shore of a bay (Fig. 1b) which penetrates approximately 1.6 km inland and is 0.8 km in width. The south eastern shore is dominated by a smelting plant which extracts nickel from laterite ore. The ore consists primarily of the oxides of silica, iron and aluminium, with smaller quantities of calcium, magnesium, chromium, nickel and cobalt and trace amounts of sulphur and arsenic. The by-products of smelting are dust, a small part of which escapes into the atmosphere and slag, which is discharged into Evoikos Gulf. Marine pollution in the bay occurs, in addition to atmospheric fall out, through spillages of the ore, coal (used in the furnaces), slag and the ferro-nickel product.

The aim of the present work was to study the metal accumulation in a variety of marine animals in order to determine their suitability as "sentinel species" for the assessment of pollution by heavy metals.

2. MATERIALS AND METHODS

2.1 Description of sampling sites

Four contaminated sites were chosen at Larymna as shown in Fig. 1a and described below.

Site 1

The mediolittoral zone consists of large rocks which are stained with black coloured material up the high water mark. In the infralittoral, between

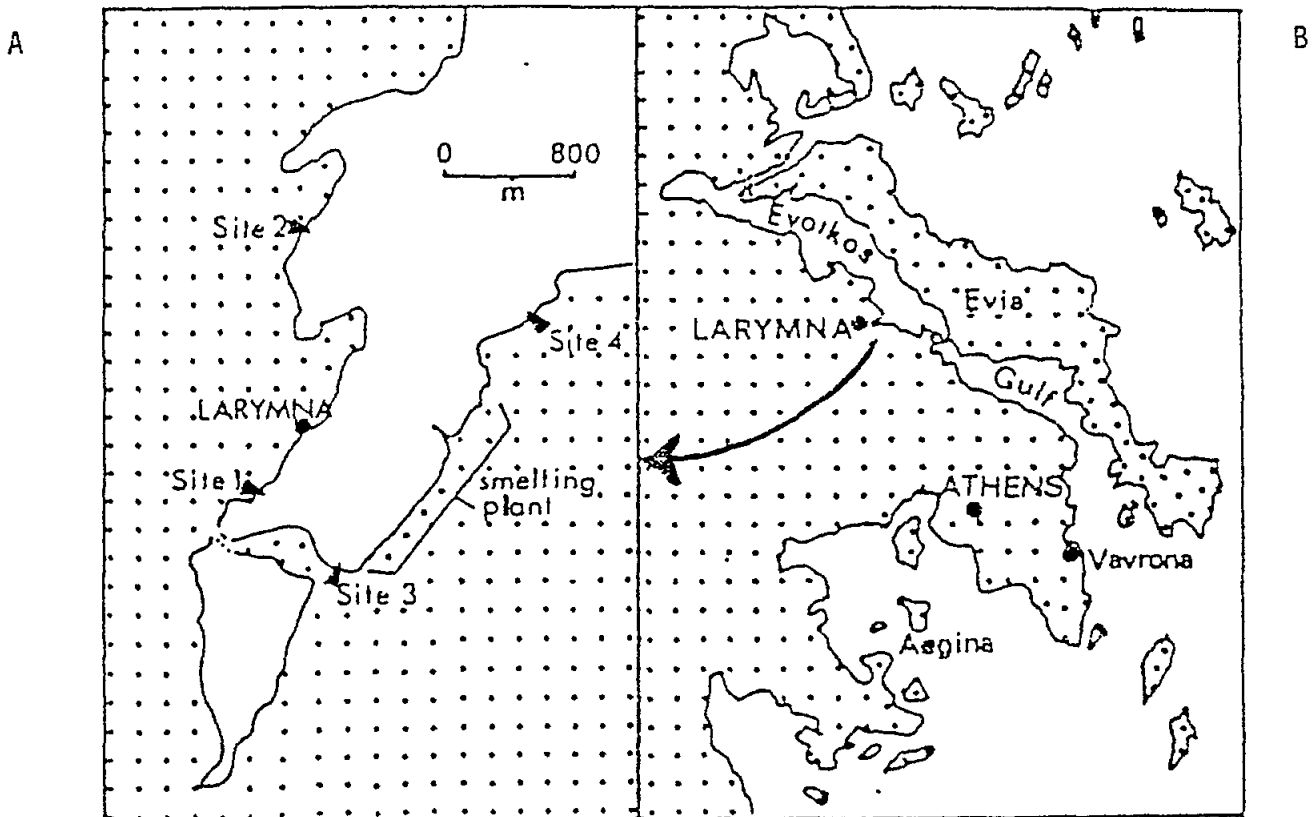


Fig. 1 Maps showing the location of Larymna on the east coast of Greece and the position of the sampling sites and smelter in the bay

occasional rocks there is muddy sand which is covered by a thin bed of *Zostera*. Associated with *Zostera* is an important population of the gastropod *Cerithium vulgatum* and an associated population of the gastropod *Murex trunculus*. Sea water has much fine sediment in suspension.

Site 2

The mediolittoral zone is covered with pebbles. The infralittoral is rocky with a short algal tuft close to the shore and a *Zostera* bed further off-shore. This site supports a variety of animals including the mediolittoral gastropod *Monodonta mutabilis* and the infralittoral *Cerithium vulgatum*. The water is much clearer than in Site 1.

Site 3

In both the mediolittoral and the infralittoral, pebbles and small rocks lie over finer sediments of red/brown colouration. A limited range of animals includes the gastropod *Monodonta articulata* and the bivalve *Mytilus galloprovincialis*. Sea water has sediment in suspension.

Site 4

A more exposed steep rocky shore, mostly bare of algae but with occasional Enteromorpha, Monodonta turbinata and Monodonta mutabilis occur in abundance. Sea water is clear of suspended sediment.

A clean control area at Vravra, in the south Evoikos Gulf provides a site with muddy sand and turbid water which supports *Zostera* and Cerithium vulgatum and an adjacent rocky shore with clear water and abundant Monodonta mutabilis. The clean site is shown in Fig. 1b.

2.2 Time of sampling

Two general surveys took place in July and November 1986 at the four sites of Larymna, during which a variety of plants and animals were collected and analysed for heavy metals.

On the basis of the preliminary results it was decided to concentrate on the study of gastropods. Thus, during the visits to Larymna which followed in January and August 1987, only gastropods were collected.

The clean site in Vravra was visited in August 1987.

2.3 Laboratory methods

The animals were brought alive to the laboratory where they were kept for one week in artificial sea water, periodically changed, to eliminate the gut contents. They were then dissected and the viscera were analysed separately from the rest of the body. In some occasions, in the preliminary surveys, the animals were analysed whole while in some others 5-8 specimens were pooled together to form one sample. The plants were washed to remove sediment.

The preparation of samples was carried out according to the methods described by Bryan et al. (1985). The sediment was sieved through a 200 µm plastic mesh with 50% sea water (salinity 34.3‰). After settling overnight, most of the water was decanted and the remaining sediment was air dried. The plant and animal tissues were dried in an oven at 80°C. Both sediment and tissues were digested in glass liquid-scintillation-counting vials with "Aristar" HNO₃. About 50 ml of acid was added to 1 g of material. The vial was then covered with a glass reflux and heated on a hot plate for 1-2 days until a yellow solution was obtained. After removing the reflux, the acid was slowly evaporated. The residue was dissolved in concentrated HCl and then diluted to obtain 10% or 1N HCl. The metals Cu, Co, Cr, Fe, Mn and Zn were analysed using a Perkin Elmer 603 flame atomic absorption spectrophotometer with a background correction for Co and Cr; carbon furnace atomic absorption was used for measuring Ni.

3. RESULTS

3.1 Uptake of metals by different invertebrates from Larymna (Results from the general surveys)

The concentrations of metals in sediment and biota from Larymna are shown in Table 1. The Table also shows for comparison the metal concentrations

Table 1
Concentrations ($\mu\text{g g}^{-1}$ dry wt) of metals in sediment and biota.

Species	Date of collection	Part of animal	Site	No. of samples	Co	Cr	Cu	Fe	Mn	Ni	Zn
<i>Actinia equina</i>	Jul 86	Whole	2	4	0.4 ± 0.4	1.0 ± 0.4	6.2 ± 0.4	139.1 ± 44.2	156.1 ± 27.1		273.9 ± 18.7
<i>Mytilus gallo-provincialis</i>	Jul 86	Whole	3	7	0.7 ± 0.5	3.0 ± 0.8	3.7 ± 0.6	191.7 ± 42.5	8.7 ± 3.4		67.9 ± 17.1
<i>Venerupis decussata</i>	Nov 86	Whole	1	3	3.1 ± 2.2	4.6 ± 0.6	9.1 ± 5.4	335.6 ± 160.5	9.4 ± 3.0	13.6	66.2 ± 4.3
<i>Pinna nobilis</i>	Nov 86	Muscle	2	1	0.8	10.2	7.4	348.6	436.1		144.8
<i>Cerithium vulgatum</i>	Nov 86	Whole	1	10	104.6 ± 35.9		71.0 ± 21.2	1898.4 ± 479.6	2131.0 ± 759.9	187.3 ± 94.3	2238.9 ± 561.4
	Jul 86	Viscera	1	1P ^a	75.8		71.1	1024.8	1396.3		4584.9
	Jul 86	Viscera	2	1P	27.9	102.9	35.9	2091.1	1307.0		2051.0
<i>Monodonta articulata</i>	Nov 86	Whole	3	10	1.4 ± 0.5	11.2 ± 2.1	125.4 ± 37.3	1360.1 ± 333.6	23.8 ± 13.9	10.5 ± 4.3	102.0 ± 19.4
	Jul 86	Viscera	3	2P	0.7	51.1 ± 1.9	214.3 ± 13.9	3661.8	64.6 ± 12.1		93.8 ± 4.0
<i>M. mutabilis</i>	Nov 86	Whole	4	10	1.1 ± 0.3	4.6 ± 1.9	136.6 ± 25.1	610.0 ± 142.2	39.5 ± 11.0	5.9 ± 0.9	98.6 ± 20.3
	Jul 86	Viscera	4	3P	1.1 ± 0.7	7.4 ± 2.5	161.6 ± 30.6	1453.0 ± 70.1	49.4 ± 12.1		65.8 ± 9.1
	Jul 86	Viscera	2	2P	1.7 ± 0.7	106.2 ± 25.7	119.5 ± 90.4	4771.5 ± 971.0	33.6 ± 8.4		90.0 ± 10.1
<i>M. turbinata</i>	Nov 86	Whole	4	10	2.3 ± 1.1	5.6 ± 2.4	109.1 ± 15.0	660.3 ± 216.6	36.4 ± 7.6	12.0 ± 0.2	65.7 ± 11.3
	Jul 86	Viscera	4	5P	3.1 ± 1.4	9.5 ± 5.8	128.9 ± 35.4	1073.0 ± 357.4	73.9 ± 23.5		90.8 ± 8.4
<i>Conus mediterraneus</i>	Nov 86	Whole	1	3	3.0 ± 0.8	1.0 ± 0.2	273.3 ± 57.8		10.1 ± 2.00	10.8 ± 4.1	121.3 ± 11.1
<i>Cypridocca leana</i>	Jul 86		1	1P	9.6	5.6		694.1	650.9		135.3
<i>Cypridocca roots</i>	Jul 86		1	1P	1.2	5.1	10.4		33.2		69.3
<i>Enteromorpha</i>	Jul 86		4	1P		7.7	26.5	1526.9	22.2		44.5
"Epiphytes"	Jul 86		2	1P		138.9	14.2		974.2		176.6
Sediment (total metals)	Nov 86		1	4	33.3 ± 10.0	746.0 ± 146.0	13.9 ± 1.2		327.0 ± 6.9	600.0 ± 206.0	128.0 ± 2.1
Sediment (10% HCl extract)	Nov 86		1	2	14.8 ± 1.8	277.1 ± 17.5	8.7 ± 3.0		253.6 ± 9.8	127.5 ± 25.1	53.8 ± 21.0
% metal extracted with 10% HCl					44.4	37.1	62.6		77.5	21.1	42
Slag in smelter ^b					93	3130	9	20.50%	2330	1200	50
Average Aegean sediment ^c						49	13	1.3%	280	28	35
Average in Greek Coastal regions ^d					14	136	21	21.1% _{op}	878	105	52

^aP = pooled samples.

^bVoutsinou-Taliadouri and Varnavas, 1987.

^cSmith and Cronan, 1975.

^dVoutsinou-Taliadouri et al., 1987.

in the slag in the smelter and the average in sediments from Aegean and other coastal Greek areas, as mentioned in the literature.

It seems that the sediment in the Bay of Larymna is contaminated by the activities of the smelter. However, only 21% of the Ni and 37% of the Cr is extracted from the sediment with 10% HCl, which suggests that a large proportion of these metals may not be readily available to organisms.

The concentrations of metals vary in the different species of invertebrates. The gastropod molluscs tend to concentrate metals more than the bivalve molluscs. Cerithium vulgatum shows particularly high concentrations of Co, Mn, Ni and Zn. The cone shell Conus mediterraneus has the highest concentration of Cu together with the winkles Monodonta spp., which also show a high concentration of Cr. The filter feeding bivalves Mytilus galloprovincialis and Venerupis decussata and the anemone Actinia had relatively low metal concentrations.

3.2 Differential uptake of metals by the gastropods

The concentrations of metals in different gastropods collected at various times are shown in Table 2. It can be seen that Cerithium vulgatum, as already indicated by the preliminary surveys, had much higher concentrations of certain metals than the other gastropods at all times. Murex trunculus exhibited the highest concentrations of Cd and Cu.

The relationship between the concentrations of metals in the viscera and the muscles of the four gastropods is expressed by the average viscera/muscles ratios. The estimated ratios for the invertebrates, with the 95% confidence intervals, are shown in Table 3. The asymmetry in the confidence intervals is due to the data being transformed to logarithms prior to calculations (as the variance was dependent on the mean) and then transformed back to original ratios. Paired t-tests between the values in viscera and muscle showed significant differences ($P < 0.0001$), the viscera having always higher concentrations than the muscle. Most ratios were between 4 and 7 while some exceptionally high values occurred for Mn (183), Ni (46) and Zn (63) in Cerithium vulgatum and for Cu in Murex trunculus. An exception was Cd, which occurred at higher concentrations in the muscle than in the viscera of Monodonta articulata and Monodonta turbinata while it was not significantly different in the two tissues of Cerithium vulgatum and Monodonta mutabilis. Finally, Mn was not significantly different in the tissues of Murex trunculus.

Comparisons of the concentrations of metals in animals from the polluted and clean sites were made by the Students-t test. For Cerithium vulgatum, the values for polluted sites were significantly higher than those for the clean site at a probability level of at least $P < 0.025$. Most were significant at $P < 0.001$. However, no significant differences were found for Cu at any time, or for Cd in November 1986. For Monodonta mutabilis, the concentrations of metals in specimens from Larymna were not higher than those coming from the clean site with the exception of Ni and Co ($P < 0.02$). Finally, Murex trunculus collected in Larymna had considerably higher concentrations of metals than did specimens of this species collected from the clean site. As all the species were not available at all sites and at all times, it is not possible to have a complete assessment of the spatial and temporal differences

Table 2

Concentrations ($\mu\text{g g}^{-1}$ dry wt) of metals in gastropods sampled at different dates from polluted sites (Larymna) and clean sites (Vravrona and Souvala).

Species	Date collected	Part of animal analysed	Site	No of animals measured	Cd	Co	Cr	Cu	Fe	Mn	Ni	Zn
<i>Cerithium vulgatum</i>	Nov. '86	viscera	L	10	7.98 ± 2.33	180.44 ± 63.80	40.52 ± 19.46	110.32 ± 35.80	2674.94 ± 788.04	3645.81 ± 1234.10	319.40 ± 159.01	3837.57 ± 1023.46
	Nov. '86	viscera	V	10	5.70 ± 1.72	105.39 ± 40.09	96.35 ± 27.03	142.35 ± 43.26	1861.45 ± 3204.52	1868.76 ± 639.16	101.60 ± 37.95	2213.48 ± 1503.51
	Nov. '86	muscle	L	10	8.38 ± 1.65	—	—	16.94 ± 6.68	355.59 ± 202.84	19.69 ± 10.07	5.70 ± 1.92	57.95 ± 4.37
	Jan. '87	viscera	L	10	25.79 ± 6.71	156.10 ± 95.64	36.34 ± 13.69	153.93 ± 54.71	1713.94 ± 477.62	1221.06 ± 391.44	239.86 ± 141.15	2143.02 ± 676.02
	Aug. '87	viscera	clean	10	8.67 ± 2.72	2.61 ± 0.88	7.11 ± 3.68	115.67 ± 56.39	987.92 ± 282.23	692.11 ± 193.73	31.87 ± 10.02	570.10 ± 287.96
	Aug. '87	viscera	L	10	22.80 ± 5.04	185.31 ± 97.89	40.29 ± 4.97	153.65 ± 54.42	1857.70 ± 616.85	1385.83 ± 576.32	257.41 ± 132.41	2521.65 ± 952.66
Aug. '87	viscera	V	10	18.07 ± 3.37	58.43 ± 20.80	113.60 ± 44.62	125.39 ± 44.91	1516.07 ± 791.78	1067.99 ± 364.63	49.89 ± 13.86	1652.91 ± 570.89	
<i>Monodonta articulata</i>	Nov. '86	viscera	L	10	3.69 ± 0.56	2.55 ± 0.92	20.62 ± 4.09	199.80 ± 63.86	2303.69 ± 594.87	39.48 ± 24.59	15.25 ± 6.74	107.39 ± 19.58
	Nov. '86	viscera	V	10	3.89 ± 0.85	3.40 ± 0.72	77.98 ± 29.13	195.20 ± 43.91	2934.52 ± 981.38	51.66 ± 28.43	29.13 ± 20.43	130.48 ± 11.85
	Nov. '86	muscle	L	10	5.28 ± 0.83	—	—	36.63 ± 11.93	238.82 ± 73.64	9.96 ± 5.58	4.61 ± 1.74	95.54 ± 30.21
	Jan. '87	viscera	L	10	4.52 ± 1.07	3.27 ± 0.88	23.12 ± 6.67	71.21 ± 30.03	2428.06 ± 375.95	23.20 ± 7.20	10.32 ± 3.87	113.66 ± 12.23
	Aug. '87	viscera	L	10	4.80 ± 0.71	3.71 ± 0.54	101.73 ± 35.38	243.82 ± 64.67	3767.13 ± 1176.94	6.15 ± 1.84	21.10 ± 5.49	108.00 ± 9.55
	Aug. '87	viscera	V	10	5.11 ± 1.31	3.47 ± 2.01	24.69 ± 8.46	301.32 ± 74.83	2560.91 ± 568.62	86.47 ± 51.35	50.42 ± 4.46	101.10 ± 10.43
<i>Monodonta tuberculata</i>	Nov. '86	viscera	L	10	2.95 ± 1.14	2.10 ± 0.55	9.02 ± 4.16	222.80 ± 42.06	1030.83 ± 279.57	67.08 ± 19.66	9.29 ± 1.46	88.63 ± 11.67
	Nov. '86	muscle	L	10	3.62 ± 0.51	—	—	43.15 ± 9.60	158.87 ± 28.12	9.42 ± 1.66	2.15 ± 0.45	108.95 ± 36.71
	Aug. '87	viscera	clean	10	5.77 ± 0.98	1.98 ± 0.45	25.17 ± 11.56	222.55 ± 83.55	3632.14 ± 1280.28	16.79 ± 6.11	6.46 ± 1.67	135.25 ± 20.12
	Aug. '87	viscera	V	10	2.63 ± 0.46	2.62 ± 0.65	9.07 ± 4.07	159.08 ± 37.16	1897.11 ± 634.68	11.46 ± 2.03	17.21 ± 5.46	85.03 ± 17.54
<i>Monodonta turbinata</i>	Nov. '86	viscera	L	10	1.53 ± 0.54	4.30 ± 1.97	10.29 ± 4.39	194.02 ± 24.75	1235.42 ± 398.46	58.16 ± 15.72	11.21 ± 0.30	110.39 ± 20.55
	Nov. '86	muscle	L	10	1.08 ± 0.46	—	—	39.89 ± 11.43	123.42 ± 12.35	10.08 ± 4.27	1.76 ± 0.30	66.40 ± 9.78
	Aug. '87	viscera	L	10	4.33 ± 1.19	1.97 ± 1.90	6.68 ± 3.82	157.39 ± 65.05	1231.18 ± 541.50	20.95 ± 7.47	18.39 ± 4.51	113.38 ± 24.22
<i>Conus mediterraneus</i>	Nov. '87	viscera	L	4	9.34 ± 3.39	5.82 ± 2.28	0.67 ± 0.32	497.75 ± 175.16	—	11.33 ± 2.52	11.00 ± 4.24	220.75 ± 53.03
<i>Patella caerulea</i>	Jul. '86	viscera	L	3	—	0.52 ± 0.11	6.15 ± 0.80	77.10 ± 3.53	—	8.09 ± 4.85	—	180.04 ± 11.09
<i>Nuxia truncifus</i>	Nov. '87	viscera	L	9	61.98 ± 14.99	5.25 ± 1.37	14.71 ± 6.62	3450.05 ± 1818.18	959.59 ± 453.43	13.97 ± 5.08	26.81 ± 10.42	3441.81 ± 968.66
	Nov. '87	muscle	L	9	1.53 ± 0.13	0.70 ± 0.15	1.48 ± 0.41	20.20 ± 4.99	118.83 ± 43.54	6.75 ± 2.62	1.08 ± 0.24	138.54 ± 23.93
	Mar. '88	viscera	clean	10	—	1.69 ± 1.88	0.61 ± 0.17	156.89 ± 88.80	104.10 ± 49.40	3.90 ± 0.90	1.18 ± 0.26	512.40 ± 139.50

in the concentrations of metals. Some indications, however, are provided by a two way analysis of variance applied to the data for Cerithium vulgatum collected from Sites 1 and 2 in November 1986 and August 1987 (Table 4a) and for Monodonta articulata collected from Sites 2 and 3 at the same time (Table 4b). The concentrations of metals other than Cu in Cerithium vulgatum were significantly different between the two sites. Specimens from Site 1 had higher concentrations (see Table 2) than those from Site 2, except for Cr which was found in higher concentrations in specimens from Site 2. Differences in time were observed for Cd, Fe, Mn and Zn. Cd concentrations were significantly higher in August, while Fe and Mn levels were higher in November. Comparisons of metals in Monodonta articulata showed that most concentrations were significantly different between sites, with specimens from Site 2 having higher concentrations than those from Site 3 (see also Table 1). The concentrations of Cd and Cu did not show differences with site but differed with time. Cobalt concentrations changed both with site and time as shown by the significance in the site/date interactions; concentrations increased in specimens from one site and decreased in specimens from the other.

Table 3

Estimated viscera/muscle ratios for metal concentrations (*italics*) with 95% confidence intervals. Paired t-tests showed significant differences ($P < 0.001$) between viscera and muscle concentrations for all values except those marked with an asterisk.

Species	Cd	Cu	Fe	Mn	Ni	Zn
<u>Cerithium vulgatum</u>	0.79 <i>0.94(*)</i> 1.13	4.58 6.39 8.90	5.39 <i>8.17</i> 12.38	128.38 <i>183.09</i> 262.43	34.21 <i>46.53</i> 63.27	49.60 <i>62.80</i> 79.36
<u>Monodonta articulata</u>	0.63 <i>0.73</i> 0.84	4.35 <i>5.31</i> 6.49	8.00 <i>9.78</i> 11.94	2.32 <i>3.39</i> 4.95	2.18 <i>2.77</i> 3.53	0.90 <i>1.16</i> 1.49
<u>Monodonta mutabilis</u>	0.67 <i>0.83(*)</i> 1.03	4.52 <i>5.10</i> 5.77	5.19 <i>6.28</i> 7.62	5.12 <i>6.69</i> 8.73	2.86 <i>3.25</i> 3.70	0.69 <i>0.84(*)</i> 1.04
<u>Monodonta turbinata</u>	0.53 <i>0.61</i> 0.71	3.97 <i>4.89</i> 6.04	7.62 <i>9.55</i> 11.98	4.25 <i>5.47</i> 7.05	4.12 <i>4.44</i> 4.79	1.42 <i>1.64</i> 1.90
<u>Murex trunculus</u>	20.00 24 30	91 144 299	- - -	1 2(*) 3	6 10 16	18 24 31

In an attempt to identify factors affecting the distribution of metals in organisms from the different sites, a principal components analysis was applied to the data. Originally all the available data were included in the analysis, but the high concentrations of Cu and Cd in Murex trunculus masked all other variations, so this species was eventually excluded. The final analysis produced three axes with variance percentages of 54.7, 17.0 and 11.8.

Table 4a

Results of the two way analysis of variance for the concentrations of metals in Cerithium vulgatum from Sites 1 and 2 in November and August.

	Cd		Co		Cr		Cu		Fe		Mn		Ni		Zn	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Site (S)	11	†	25	‡	52	‡	<1	N.S.	4	*	16	‡	41	‡	5	*
Date (D)	163	‡	1	N.S.	<1	N.S.	1	N.S.	4	*	35	‡	3	N.S.	18	‡
S/D Interaction	1	N.S.	2	N.S.	<1	N.S.	6	*	<1	N.S.	8	†	<1	N.S.	<1	N.S.

F = F-ratio: ratio between variances
P = Probability: N.S. not significant
*P<0.05; †P<0.01; ‡P<0.001

Table 4b

Results of the two way analysis of variance for the concentrations of metals in Monodonta articulata from Sites 2 and 3 in November and August.

	Cd		Co		Cr		Cu		Fe		Mn		Ni		Zn	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Site (S)	<1	N.S.	<1	N.S.	85	‡	2	N.S.	10	†	12	†	12	†	12	†
Date (D)	16	‡	<1	N.S.	4	N.S.	14	‡	3	N.S.	<1	N.S.	3	N.S.	11	†
S/D Interaction	<1	N.S.	4	*	2	N.S.	2	N.S.	<1	N.S.	22	‡	<1	N.S.	4	N.S.

F = F-ratio: ratio between variances
P = Probability: N.S. not significant
*P<0.05; †P<0.01; ‡P<0.001

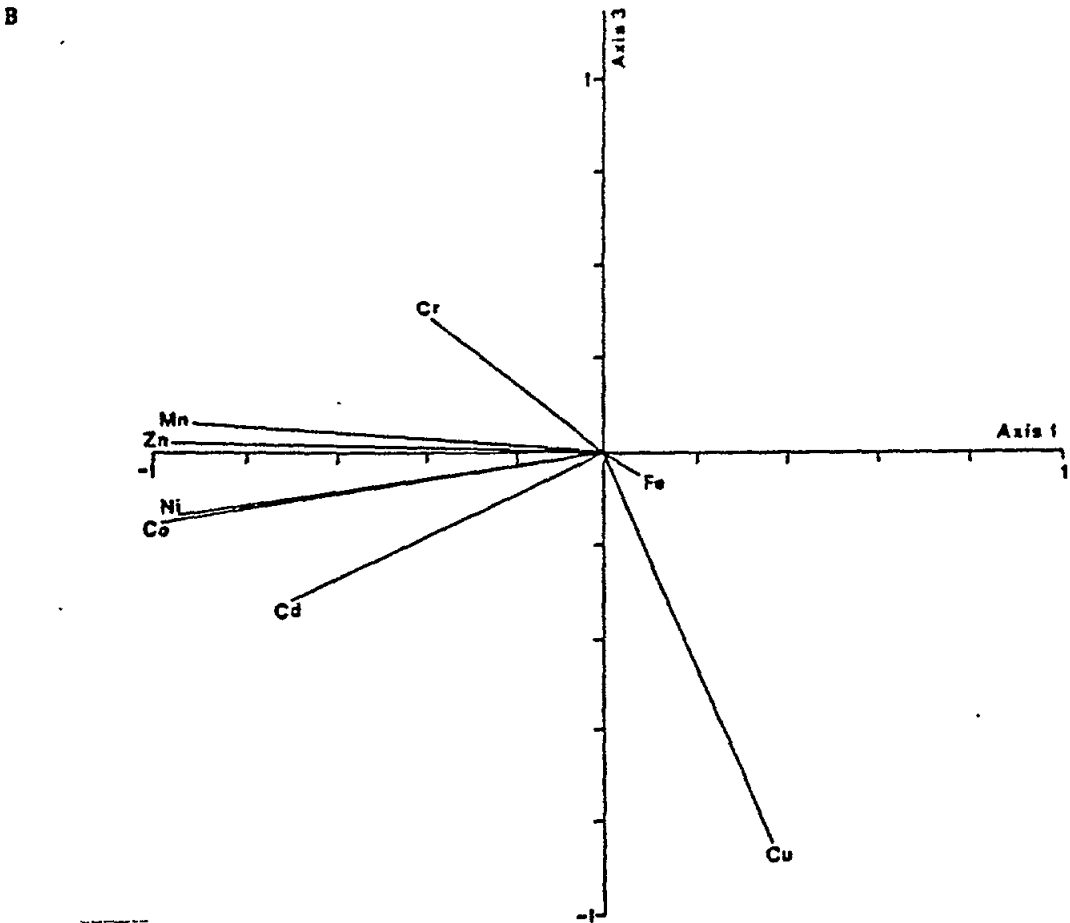
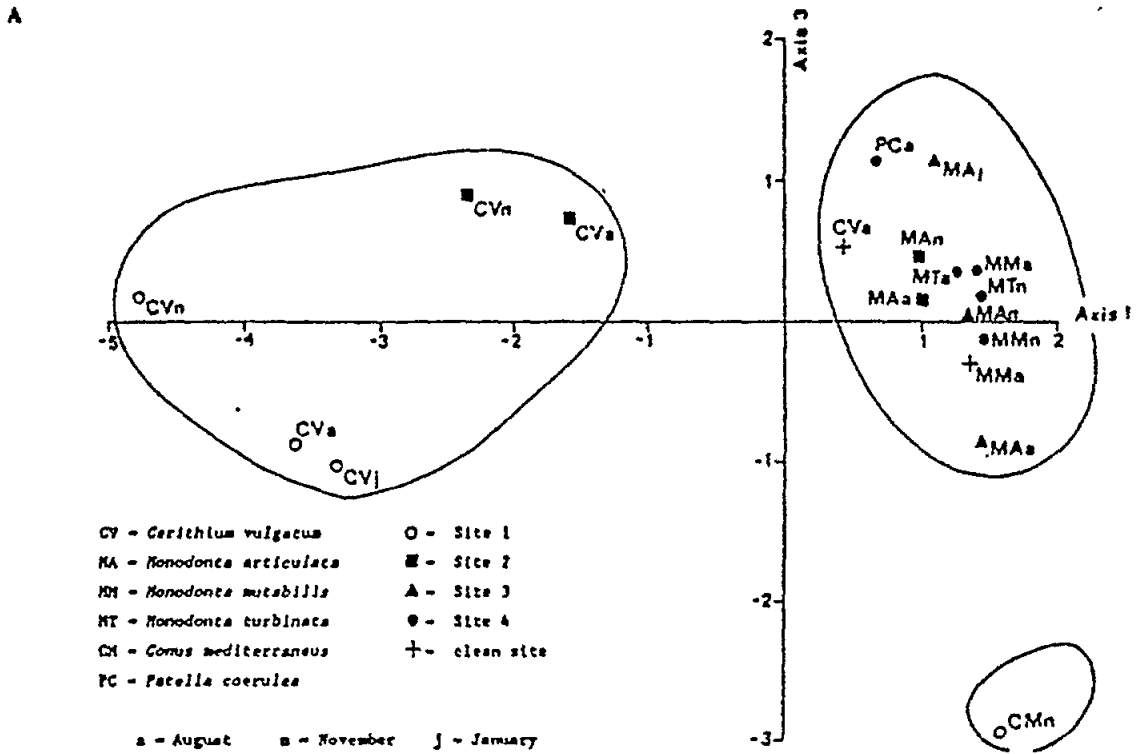


Fig. 2 Principal component analysis (see text) A = Ordination of species along Axes 1 and 2. B = Correlation coefficients of the metals

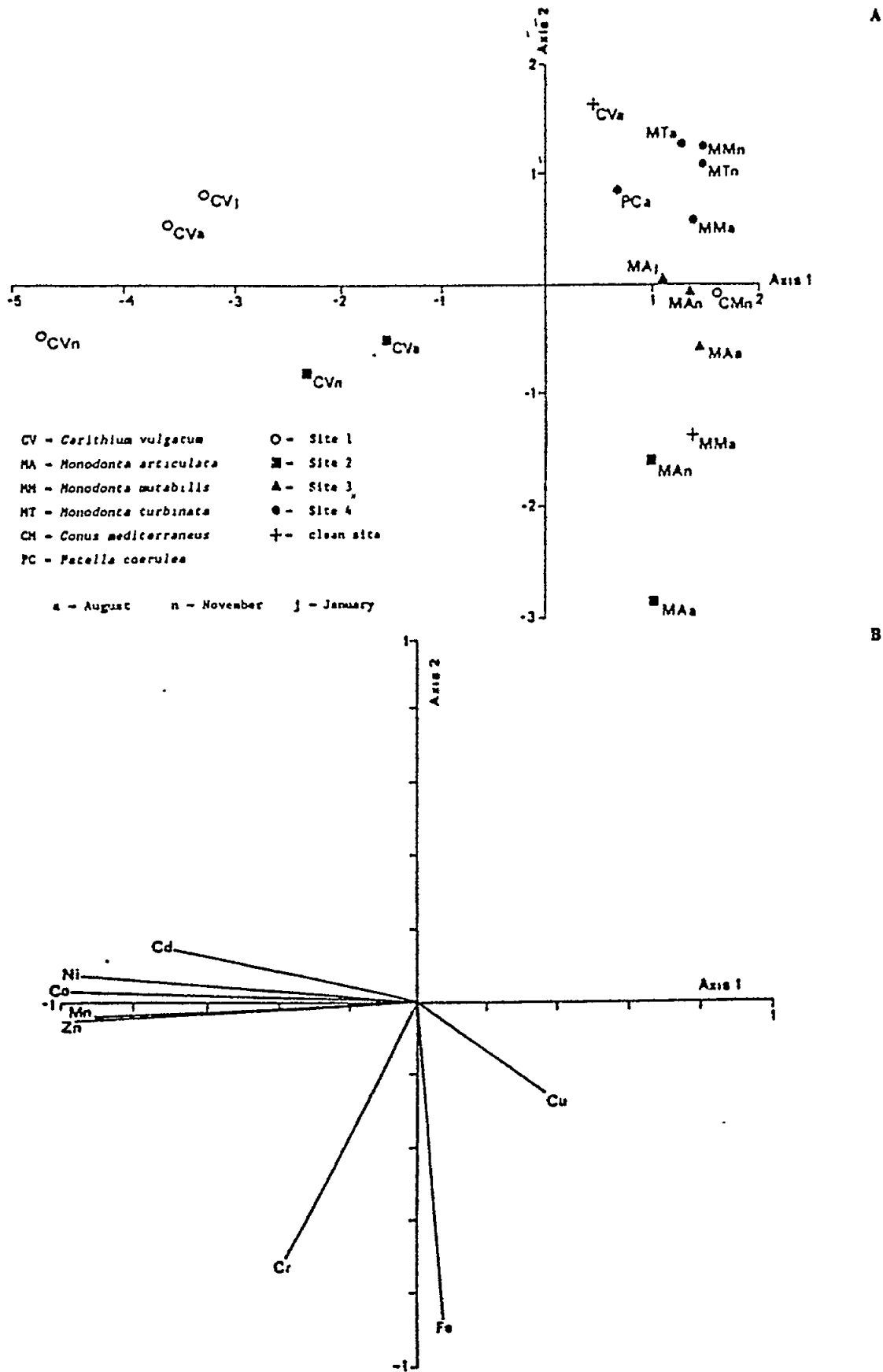


Fig. 3 Principal component analysis (see text) A = Ordination of species along Axes 1 and 3. B = Correlation coefficients of the metals

In Fig. 2A the different species are plotted on Axes 1 and 2. Cerithium vulgatum from Larymna is distinctive in consistently having low scores on Axis 1 and Cerithium vulgatum from Site 1 being lower than Site 2. All the other animals have values which are similar and higher on Axis 1. It appears that this axis represents the total concentration of metals and is related to the species. Axis 2 separates the species according to the sampling site. Species from Site 4 have the highest scores, followed by Sites 3 and 1 and finally Site 2 with the lowest scores. This sequence tends to follow the same order in which the clean sea water which enters the bay, passes the sites, starting with site 4, smelter, site 3, 1 and 2.

When Axis 1 is plotted against Axis 3, the species form into three groups (Fig. 3). Group 1 includes all the samples of Cerithium vulgatum from Larymna, Group 2 all the Monodonta species and Patella coerulea, whilst Conus mediterraneus appears on its own. These groups may represent different feeding types; Cerithium vulgatum is a deposit and detritus feeder, Monodonta spp and Patella coerulea are herbivores and Conus mediterraneus is a carnivore. Alternatively, the separation may be related to different evolutionary groups. Patella and Monodonta belong to the Archaeogastropoda, Cerithium to the Mesogastropoda and Conus to the Neogastropoda.

No seasonal trends were revealed by the principal components analysis.

4. DISCUSSION

At Larymna, the concentrations of Cd, Co, Cr, Fe and Ni in the sediments are higher than the average concentrations in clean coastal sediments (Smith and Cronan, 1975; Voutsinou-Taliadouri *et al.*, 1987) but are comparable with those found in some polluted areas in Greece. Thus, in comparison with the Bay of Kavala, which is affected by oil-platforms and a fertilizer factory on the shore (Voutsinou-Taliadouri, 1985), Larymna has increased concentrations of Ni and Co. The values in Larymna, however, are lower than those found in the Thermaikos Gulf (Voutsinou-Taliadouri and Leondaris, 1985) which is influenced by the sewage and industrial effluents from Thessaloniki, the second largest city in Greece.

Among the invertebrates, the mussel Mytilus galloprovincialis exhibits lower concentrations of heavy metals in species collected at Larymna than in mussels from other coastal regions of Greece which are polluted by sewage (Grimanis *et al.*, 1983). The carpet shell Venerupis decussata also has low metal concentrations compared with sediments from the Bay of Izmir (Tuncer and Uysal, 1982). For the anemone Actinia the levels are similar to those noted by Bryan and Gibbs (1983) for the same species from unpolluted sites in south Devon, with the exception of Mn which is more concentrated in organisms collected from Larymna. For Monodonta mutabilis, the concentrations of metals in specimens from Larymna were not higher than those in specimens obtained from the clean site in Vravra with the exception of Ni and Co.

Cerithium vulgatum was the species which showed the highest concentrations of most metals in Larymna and these (with the exception of Cu) were significantly higher than in the clean site. As shown by Nott and Nicolaidou (1989) metals in the body of Cerithium vulgatum accumulate in both the mineralised phosphate granules of the basophil cells and the lysosomal residual bodies of the digestive cells. This also explains the much higher concentration of metals

in the viscera than in the muscle (viscera to muscle concentration ratio from 6.39 for Cu to 183.09 for Mn).

Murex trunculus from Larymna showed the highest concentrations of Cu and Cd. Most of the Cu was accumulated in the viscera (viscera:muscle ratio 144). This is in accordance with the findings of Bouquegneau and Martoja (1982) for Murex brandaris in which Cu from the metabolism of haemocyanin is stored as CuS in pore cells located in the connective tissue of the digestive gland. According to these authors, this applies to Meso- and Neogastropods, which probably explains the increased concentrations of Cu in the other Neogastropod species studied here, Conus mediterraneus. Murex trunculus from Larymna also had very high concentrations of Cd in the viscera. Unfortunately, Cd values from the clean site are not available in the present study. However, since the observed values match those for the same species at a clean site given by Bouquegneau et al. (1983), it is assumed that Larymna is not highly polluted with Cd.

The relatively low concentrations of metals in the filter feeders (Mytilus galloprovincialis, Venerupis decussata) and the higher concentrations of the metals in the herbivores (Monodonta spp) and especially the detritus feeder (Cerithium vulgatum) suggest that one pathway for metal accumulation may be through the plants which also show some high concentrations, i.e. Cymodocea leaves are rich in Mn and the "epiphytes" in Cr, Mn and Zn. The relationship between metal accumulation and feeding mode of the organisms was shown for the gastropods by the principal components analysis.

Temporal variations in the concentrations of metals in the tissues of molluscs are only occasionally mentioned in the literature (e.g. Bryan, 1973). In most organisms, and particularly in bivalves, variation is attributed to tissue weight changes which are related to the reproductive cycle (Boyden and Phillips, 1981). In the present study, temporal variation was evident in only a few cases, the most important being that of Cd concentration which varied between November and August in both Cerithium vulgatum and Monodonta spp. Changes in Cd concentration in an annual cycle were observed in Murex trunculus from an uncontaminated site by Bouquegneau et al. (1988). They found that the greatest decrease in Cd concentration in the viscera occurred in the autumn and were associated not with reproduction but with the construction of a growth ring in the shell. The same could be true for Cerithium vulgatum and Monodonta spp. at Larymna, which also showed the lowest Cd concentration in November.

Fluctuations of Mn concentration occurred in the oyster Crassostrea gigas (Boyden and Phillips, 1981) and it was suggested that the changes were positively correlated with the rate of formation of the shell; it was suggested also that significant losses of Mn were associated with spawning. Large fluctuations of Mn concentration in Donax trunculus were attributed by Mauri and Orlando (1983) to both gonadal and environmental variations such as freshwater input by a nearby river. A factor which may contribute to the variability of Mn in Cerithium vulgatum is the availability of the metal in the environment. If, as suggested by Nicolaidou and Nott, (1989), the main source of Mn for Cerithium vulgatum is the Cymodocea detritus, then fluctuations in the amount of this material in the sediment may account for some of the Mn variation. Like other marine phanerogams, Cymodocea loses its leaves in the autumn, and it should be expected that more detritus would occur in the sediment at this time.

An interesting observation is that, with the exception of Zn, Cu and Cd, Murex trunculus had lower concentrations of metals than Cerithium vulgatum on which it was feeding. However, Murex trunculus accumulates Cu and Cd even in a clean environment. Lack of biomagnification has also been reported by other authors (Young, 1977; Ward *et al.*, 1986). Ireland and Wootton (1977) found lower concentrations of Zn, Mn, Cu and Pb in the gastropod Nucella lapillus than in the barnacles on which it was feeding (Ireland, 1979). The lower concentrations in the carnivores may reflect the unavailability of the detoxified metals in the prey species, as in Cerithium vulgatum, where it is found in an insoluble form within intracellular granules (Nott and Nicolaidou, 1989).

The present study showed that a number of marine gastropods are differentially selective for the accumulation of a range of metals. This must reflect the availability of the elements in the environment and the diet and digestive physiology of the animals. From the species examined, Cerithium vulgatum was the one which showed higher concentrations of metals and could be used as a "sentinel species" for metal contamination. The viscera contain particularly high concentrations, therefore analyses should be carried out on dissected specimens; this would refine the levels of detection and accuracy.

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EFFECTS OF SEWAGE ON THE DISTRIBUTION OF BENTHIC FAUNA IN THE SARONIKOS GULF (GREECE)

by

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ABSTRACT

Seasonal sampling of the benthic fauna in Saronikos Gulf was undertaken during 1989 along three transects at increasing distance from the main sewage outfall of Athens Greater Area. Additional sampling took place in the Bay of Faliro (part of Saronikos Gulf) along three transects running from the inner to the outer bay.

Based on the results of the faunistic and statistical analysis, Saronikos Gulf was divided into three zones according to the degree of pollution: a heavily polluted zone; a transitional polluted zone and a transitional but less aggravated zone. This zonation was constant throughout the sampling seasons.

In Faliro Bay the analysis showed an analogous pollution gradient along the sampling transects, the shallower stations closer to the Kifissos river mouth being the most affected. The disturbance was attributed to the organic load transported by Kifissos river and to development works along the coastal zone.

1. INTRODUCTION

Benthic communities have been widely used in pollution monitoring programmes as they reflect the degree of pollution by showing spatial and temporal variations in distribution (Pearson and Rosenberg, 1978; Gray and Mirza, 1979).

In field observations dealing with pollution effects upon a soft bottom fauna, the criteria suggested by Leppakoski (1975) include parameters such as: species richness (S), population density (N), community diversity (H), species diversity (d) and evenness of distribution (J). Changes of the above parameters are indicators of environmental changes in the area and can be used to assess the degree of pollution.

The work of Peres and Picard (1958) provide us with a description of some benthic communities in the Piraeus shore before the construction of the Central Sewage System.

After construction, the benthos of the Saronikos Gulf was studied by Vamvakas (1970) and Zarkanellas and Bogdanos (1977) who examined the state of the benthic communities in the upper Saronikos Gulf. A detailed study of the benthic community structure in Elefsis bay, carried out in 1985, revealed that the azoic zone has expanded to include stations which were not previously azoic (Zenetos and Bogdanos, 1987). Other studies in the area are those of

Diapoulis (1983) (phytobenthos) and Panayotidis (1988) (marine phanerogams). The sediments were studied by Schwarts and Tziavos (1975) and by Anastasakis (1984).

A pilot study concerning the benthic communities in the entire Saronikos Gulf was carried out by our Institute in 1987. Stations were sampled along three transects, on the west, central and eastern parts of Saronikos Gulf. Results showed that there is a gradient in the community density along the transects (Zenetos et al., 1990). However, the communities were not studied in detail.

The main objective of the proposed study was to examine the state of the benthic communities, in order to determine the seasonal variations of the biological parameters (S, N, H, d, J) and to evaluate the degree of pollution in the area. Furthermore, the study of the present condition of the benthic populations in the Saronikos Gulf may be of use as a reference study, given the fact that a new outfall of the Central Sewage System is under construction.

The project was extended to include Faliro Bay, a shallow area affected by two additional sources of disturbance: a) the organic load of industrial origin transported into the sea by the Kifissos river (at present restricted to a drainage canal) and b) the urban development works along the coastal zone of the bay which cause considerable perturbation of the hydrodynamic equilibrium around the construction site.

2. MATERIALS AND METHODS

2.1 The study area

The Saronikos Gulf (Aegean Sea) is divided into two sectors (west and east) by a series of islands and peninsulas and shows low water masses with different hydrological characteristics. The western sector is semi-enclosed, while the eastern sector communicates freely with the Aegean Sea.

Coachman et al. (1976), in a study of the hydrology of the Saronikos Gulf described a third water mass in the central part of the Gulf (Inner Gulf). In this sector the surface water temperature ranges from 14EC (January-February) to 27EC (July-August), while at 75 m. depth the temperature varies between 38EC and 39EC (Zodiatou et al., 1988).

The underwater outfall of the Central Sewage System of domestic wastes of the city of Athens is found at Keratsini, near the port of Piraeus. Constructed in 1959, the outfall discharges on average 0.5×10^6 tons of untreated effluents into the sea and is considered as the main source of pollution of the Saronikos Gulf (Friligos, 1981a; 1981b).

Sampling in the Saronikos Gulf was carried out along three transects at the western, central and eastern parts of the Gulf (Fig. 1)

In the Bay of Faliro the sampling stations were also situated along three transects (western, central and eastern) at increasing distances from the mouth of the Kifissos river (Fig. 2).

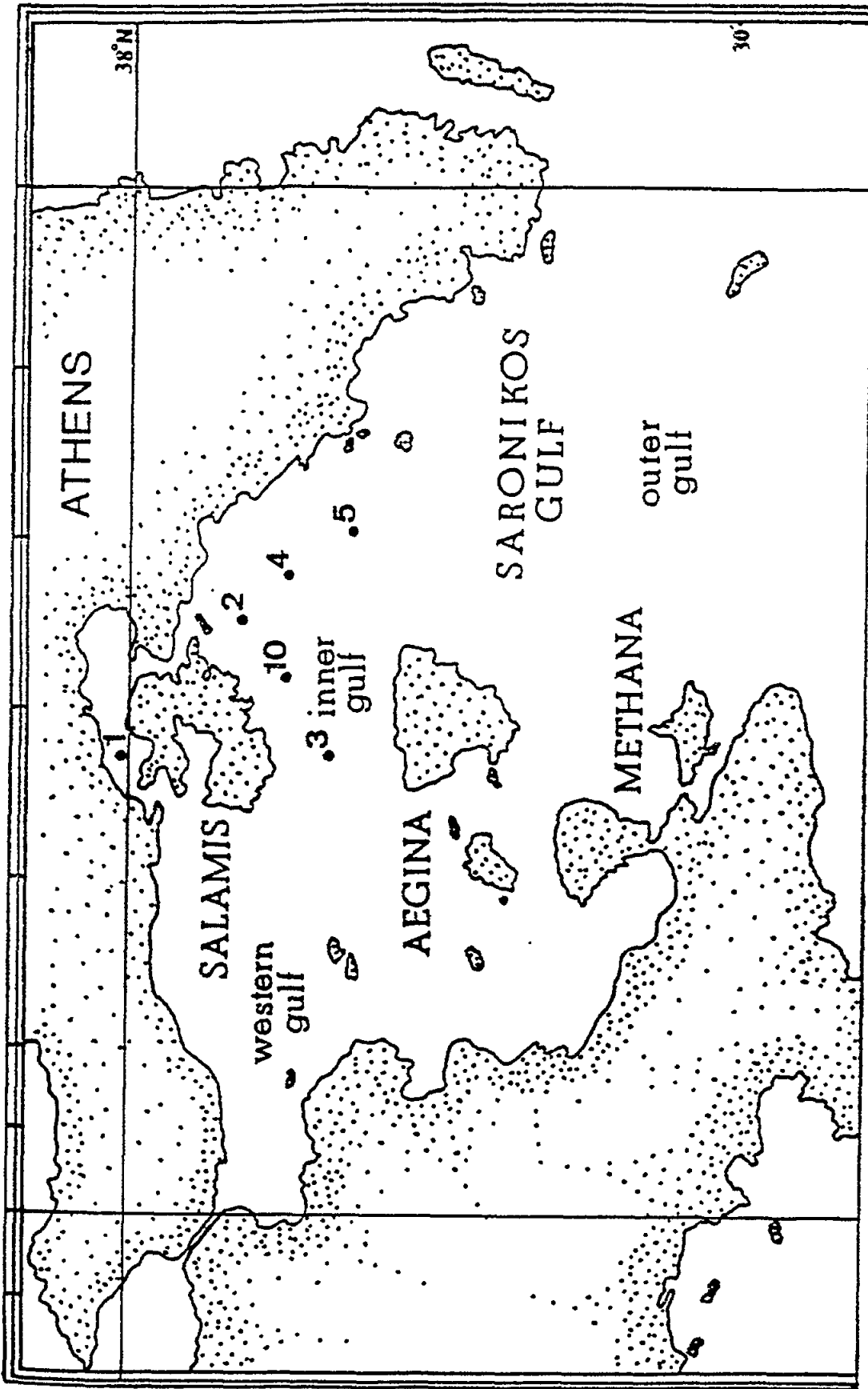


Fig. 1 Sampling sites in the Saronikos Gulf

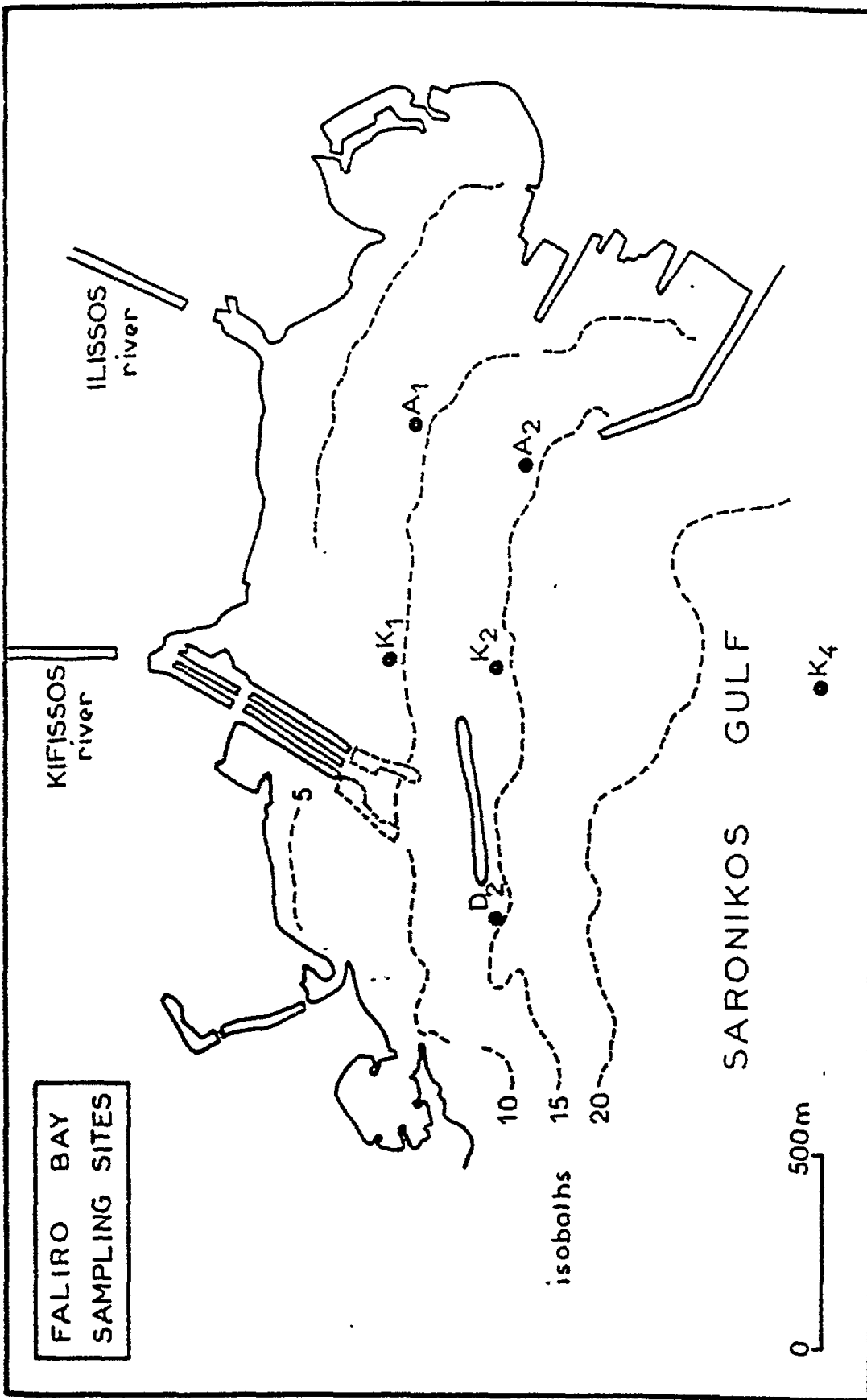


Fig. 2 Sampling sites in Faliro Bay

2.2 Sampling - Statistical treatment of the samples

Sampling in the Saronikos took place during 4 cruises in March, June, November 1989 and in April 1990. In April samples were collected from station 2 instead of station 1. The sampling equipment included a 0.05 m² Ponar grab. The aim was to collect five samples at each station but where this was not attainable, fewer samples were collected.

Sampling in Faliro Bay took place during April and June 1991. The same sampling equipment and methodology was used as for the Saronikos Gulf. Six stations were sampled in April. For four of these stations sampling was repeated in July.

The depth of the stations and the number of the samples at each station in Saronikos Gulf are shown in Table 1. Part of the material collected was retained for sediment analysis which was conducted by the Geological laboratory of our Institute. The sediment type was characterized according to Folk (1954). The results obtained are shown in Table 2. Depth and sediment characteristics in the Bay of Faliro sampling sites are shown in Table 3.

Table 1

Depth of stations and number of samples in the Saronikos Gulf.

Station	Depth (m)	Number of samples			
		2/89	6/89	11/89	4/90
1	30	5	4	4	-
2	83	-	-	-	2
3	90	5	4	4	4
4	72	3	4	5	5
5	85	2	4	4	5
10	90	2	4	5	4

Table 2

Grain size distribution in the Saronikos Gulf.

Station	Sediment type	% sand	% silt	% clay
1	muddy silt	-	-	-
2	sandy mud	19	49.5	31.5
3	muddy sand	53.1	23.2	23.7
4	muddy sand	70.7	12.6	16.7
5	muddy sand	62.1	21.2	16.7
10	mud	5.5	56.5	38

Table 3

Depth and sediment characteristics in the Bay of Faliro sampling sites.

Station	Depth (m)	Sediment type	% sand	% silt	% clay
A1	10	silt	3.9	78.6	17.5
A2	15	silt	7.5	65.3	27.2
K1	10	silt	6.7	68.4	24.9
K2	15	sandy silt	10.4	64.8	24.8
K4	25	sandy silt	19.1	46.6	34.3
D2	15	sandy silt	11.5	59.5	29

The samples were sieved on board the research vessel through a 1 mm sieve and stored in 4% formalin solution stained with Rose Bengal. Upon return to the Laboratory the organisms were sorted out from the samples and classified into the major invertebrate groups (Polychaeta, Mollusca, Crustacea and Echinodermata). Organisms belonging to the remaining invertebrate groups (Sipuncula, Anthozoa, Porifera, Bryozoa, Nemertinea etc.) were collectively placed into a separate category as Miscellaneous. The individuals were counted and identified to species level.

Community diversity was calculated using the Shannon-Wiener index (H) (Shannon and Weaver, 1963), while the species diversity (d) was determined by using the Margalef index (Margalef, 1968). Evenness was calculated using the Pielou index of evenness J (Pielou, 1969)

The raw data, expressed as the number of individuals m^{-2} (mean value of the replicate samples at each cruise) was transformed using the transformation $Y_{ji} = \log(x_{ji} + 1)$ (Field *et al.*, 1982). The Bray-Curtis similarity matrix (Bray and Curtis, 1957) was used and classification was performed on the similarity matrix using the Group Average clustering technique (Sokal and Sneath, 1963). All multivariate analyses were performed using the software package "PRIMER" developed at the Plymouth Marine Laboratory, U.K.

3. RESULTS

3.1 Macrofauna

In the Saronikos proper, for a total sampling surface of 3.95 m^2 , 9486 individuals belonging to 250 taxonomical units were examined. These consist of polychaetes, 147 species (58.8%), molluscs, 54 species (21.6%), crustaceans, 20 taxonomical units (8%), echinoderms, 9 species (3.6%) and 20 taxonomical units belonging to various minor groups, namely Anthozoa, Porifera, Bryozoa, Sipuncula etc). The distribution of species, expressed as the number of individuals m^{-2} , at each station during all sampling cruises is shown in Table 4.

Table 4

Number of individuals per m² at each station for all cruises in the Saronikos Gulf.

	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5		STATION 10					
	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90
POLYCHAETA																
<i>Amaea trilobata</i>	0	0	0	0	4	10	0	0	7	0	0	0	0	0	0	0
<i>Ampharete acutifrons</i>	0	0	0	30	20	25	25	12	27	5	20	12	0	0	0	0
<i>Amphicteis gunneri</i>	0	0	0	0	4	0	5	4	7	10	4	0	10	10	0	0
<i>Amphictene capensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ampharetidae sp.</i>	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
<i>Amphictene capensis</i>	0	0	0	0	0	0	10	0	13	0	16	0	0	0	0	0
<i>Amphinomidae sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aphroditidae sp.</i>	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Ancistrosyllis groenlandica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
<i>Arabella iricolor</i>	0	0	0	0	0	5	0	0	13	5	0	0	0	0	0	0
<i>Aricidea fauveli</i>	0	0	0	10	8	0	10	4	7	0	16	4	10	0	0	10
<i>Aricidea fragilis mediterranea</i>	0	0	0	0	4	5	5	8	0	5	0	8	10	0	15	0
<i>Aricidea simplex</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aricidea sp.</i>	0	0	0	10	4	0	0	0	0	0	0	0	0	5	0	0
<i>Arandia cirrosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
<i>Arandia sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
<i>Asychis biceps</i>	0	0	0	0	80	70	115	156	107	0	156	48	0	75	50	52
<i>Asychis gotoi</i>	0	0	0	0	64	65	35	24	7	0	24	0	0	20	0	8
<i>Bhawania reyssii</i>	0	0	0	0	0	0	0	24	0	30	0	24	10	10	85	20
<i>Branchioma vesiculosum</i>	0	0	0	0	0	0	0	0	7	0	0	0	0	0	10	0
<i>Capitella capitata</i>	4	0	321	0	0	0	0	0	7	0	0	0	0	0	0	0
<i>Chaetopteridae sp.</i>	0	0	0	0	0	0	5	0	7	0	0	0	0	0	0	0
<i>Chaetozone setosa</i>	0	0	0	2920	164	670	185	264	313	430	264	368	0	70	45	72
<i>Chloea venusta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chloemidae sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chone collaris</i>	0	0	0	0	0	0	5	4	0	0	4	0	0	0	0	0
<i>Chone filicaudata</i>	0	0	0	0	4	0	0	0	53	0	0	40	20	25	15	36
<i>Cirratulus filiformis</i>	0	0	0	0	12	0	10	16	7	10	16	0	20	5	10	0
<i>Cirrophorus branchiatus</i>	4	0	0	20	4	15	25	4	33	5	4	28	10	20	30	24
<i>Cirrophorus harpagonus</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Cossura coasta</i>	0	0	0	0	4	0	0	0	0	0	0	0	0	5	10	0
<i>Dasybranchus caducus</i>	0	0	0	0	8	0	20	24	20	10	24	40	20	70	5	44
<i>Ditrupa arietina</i>	0	0	0	0	0	0	0	4	0	140	4	4	0	5	0	12

	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5		STATION 10					
	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90
MOLLUSCA																
<i>Cingula proxima</i>	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
<i>Clausinella fasciata</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Corbula gibba</i>	304	70	1396	10	4	0	5	15	60	8	16	0	0	5	0	8
<i>Cultellus adriaticus</i>	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
<i>Cuspidaria costellata</i>	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
<i>Cylindrina cylindracea</i>	0	0	0	10	0	0	5	0	5	12	4	0	0	0	0	0
<i>Diplodonta brocchi</i>	0	0	0	0	12	0	0	7	5	0	0	0	0	0	0	0
<i>Eulimella scillai</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Falcidens gutturosus</i>	0	0	0	0	12	20	40	15	27	20	36	100	10	65	60	44
<i>Gasteropoda sp.</i>	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
<i>Gouldia minima</i>	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	4
<i>Hiatella arctica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydrobia sp.</i>	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
<i>Hinia sp.</i>	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kellyella miliaris</i>	0	0	0	0	0	20	10	0	0	10	0	0	0	10	5	4
<i>Laevicardium oblongum</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Leptaxinus ferruginosus</i>	0	0	0	0	0	40	25	40	0	0	28	4	0	25	0	0
<i>Lepton nitidum</i>	0	0	0	0	0	35	30	10	7	5	4	32	0	20	0	4
<i>Lima loscombei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
<i>Loripes lacteus</i>	0	0	0	0	0	0	0	0	0	5	0	0	0	0	5	0
<i>Macoma balaustina</i>	0	0	0	0	0	8	0	10	7	10	0	4	0	0	0	8
<i>Montacuta substriata</i>	0	0	0	0	0	0	0	0	0	15	0	0	0	10	10	0
<i>Myrtea spinifera</i>	0	0	0	50	40	95	105	70	87	75	76	100	0	20	0	24
<i>Mysella bidentata</i>	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
<i>Mysia undata</i>	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Natica alderi</i>	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Neolepton obliquatum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0
<i>Nucula hanleyi</i>	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	4
<i>Nucula nucleus</i>	0	0	0	0	0	0	0	0	20	0	0	8	0	0	0	4
<i>Nuculana fragilis</i>	0	0	0	0	24	0	50	35	0	5	8	56	0	5	10	28
<i>Nuculana pella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
<i>Odostomia conoidea</i>	0	0	0	10	0	0	0	5	0	0	0	0	0	0	0	0
<i>Parvicardium exiguum</i>	0	0	0	0	0	0	15	0	0	0	4	0	0	0	0	0
<i>Parvicardium nodosum</i>	0	0	0	0	0	0	0	0	7	0	0	0	20	0	0	0
<i>Parvicardium scabrum</i>	0	0	0	0	0	5	60	5	0	15	36	44	0	25	35	12

	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5		STATION 10					
	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90
MOLLUSCA																
<i>Phaxas pellucidus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Philine aperta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pinna nobilis</i>	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pitar rudis</i>	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
<i>Tellima ferruginosa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tellina donacina</i>	0	0	0	0	4	5	0	0	15	0	0	0	0	0	0	0
<i>Tellina nitida</i>	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
<i>Tellina pulchella</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Tellina serrata</i>	0	0	0	0	8	10	5	0	20	25	4	0	0	10	0	4
<i>Leptaxinus incrassatus ?</i>	0	0	0	0	0	0	510	0	0	0	364	0	0	0	0	576
<i>Thracia papyracea</i>	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0
<i>Thracia sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
<i>Thyasira flexuosa</i>	0	0	0	510	128	620	530	295	60	50	424	92	0	5	5	430
<i>Timoclea ovata</i>	0	0	0	0	32	5	10	55	13	350	12	76	80	85	20	28
<i>Turbonilla lactea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Veneridae</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Venerupis aurea</i>	4	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Venus sp. juv.</i>	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
CRUSTACEA																
<i>Amphipoda</i>	0	0	0	0	8	0	0	0	67	0	0	0	10	0	0	0
<i>Amphipoda sp.1</i>	0	0	0	0	0	30	20	15	0	210	24	56	0	60	25	28
<i>Amphipoda sp.2</i>	0	0	0	0	0	0	0	10	0	5	0	20	0	0	5	4
<i>Amphipoda sp.3</i>	0	0	0	0	0	0	0	10	0	0	0	24	0	5	0	0
<i>Amphipoda sp.4</i>	0	0	0	10	0	0	10	0	0	0	8	36	0	5	0	0
<i>Anomura</i>	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
<i>Anthura gracilis</i>	0	0	0	0	0	0	0	5	47	35	12	28	0	15	0	0
<i>Brachiura</i>	0	0	0	0	0	0	5	5	7	0	4	0	0	10	5	32
<i>Copepoda</i>	0	0	0	0	4	5	0	0	0	0	4	0	0	0	5	0
<i>Cumacea</i>	0	0	0	0	0	10	10	0	0	10	16	4	0	0	0	0
<i>Decapoda</i>	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0
<i>Decapoda larvae</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	4
<i>Isopoda</i>	0	0	0	0	0	0	0	0	0	0	0	4	0	5	15	12
<i>Leptocheilia savignyi</i>	0	0	0	0	0	0	5	0	53	10	0	0	0	20	15	0
<i>Macroura sp.1</i>	0	0	0	0	12	0	0	25	0	15	0	0	0	10	10	0

	STATION 1		STATION 2		STATION 3		STATION 4		STATION 5		STATION 10					
	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90	3/89	6/89	11/89	4/90
MISCELLANEA																
Nematoda	0	0	0	0	12	10	10	0	100	15	8	0	10	40	80	32
Nemertinea	0	0	3	80	8	15	5	15	13	10	12	12	20	10	15	4
Onchosesoma steenstrupi	0	0	0	0	8	25	30	15	7	10	8	16	0	5	5	8
Phascolion strombi	0	0	0	10	0	10	5	0	0	10	0	0	0	10	20	20
Phoronis muelleri	0	0	0	0	0	10	0	0	0	0	0	0	0	5	0	0
Podocoryna carnea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Porifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Priapulida	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
Sipuncula	0	0	0	10	0	30	30	15	27	0	4	8	60	0	10	4
Turbellaria	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0
Species richness S	10	1	13	41	82	66	90	78	78	80	80	96	36	95	106	99
S total	(18)			(41)	(157)				(167)				(191)			
Population density N m ²	1144	70	1939	4800	1652	2780	3305	2705	3210	3060	2505	2900	710	2265	1910	2180
N mean	(1051)			(4800)	(2610)				(2919)				(1766)			
													28	42	35	38
													(73)			
													2240	1730	5275	1412
													(2665)			

In Faliro Bay, for a total sampling surface of 1.4 m², 3876 individuals were identified belonging to 133 taxonomic units. Of these, 81 species were polychaetes (60.9%), 33 were molluscs (24.8%), 10 were crustaceans (7.5%), 3 were echinoderms (2.2%) and 7 belonged to minor taxa. The distribution of species, expressed as the number of individuals m⁻², at each station for all cruises is shown in Table 5.

3.2 Seasonal variations

3.2.1 Saronikos Gulf

Great seasonal variations were recorded at station 10 where the minimum number of species was observed in the winter (28 species) and the maximum in the summer (42 species). The respective density values were 2240 ind. m⁻² (March 89) and 5275 ind. m⁻² (June 89). The percentage of the number of species and individuals for each benthic group and each sampling station is shown in Tables 6a and 6b.

It is worth noting that the percentage of the number of species for each taxonomical group followed a relatively high range of variation over the various sampling periods at each station. The range of this variation was: for the polychaetes from 0% (June 89) to 62% (November 89) at station 1 and from 28% (November 89) to 65% (June 89) at station 10; for the molluscs from 20% (March 89) to 100% (June 89) at station 1 and 8% (November 89) at station 5; for the crustaceans from 7% (March 89) to 56% (November 89) at station 10; and for the echinoderms from 1% (March 89) to 5% (November 89) at station 4.

Respectively, the number of individuals in each major invertebrate group ranged for the polychaetes from 27% (11/89, station 1) to 85% (6/89, station 10); for the molluscs from 7% (11/89, station 5) to 100% (6/89, station 1); for the crustaceans from zero (6/89, station 1) to 13% (6/89, station 4); and for the echinoderms from zero (stations 1, 2 and 10 throughout the sampling periods) to 3% (3/89 and 11/89, station 5).

3.2.2 Faliro Bay

Negligible to very little seasonal variations were observed in the number of species of each taxonomic group at stations A2 and D2. On the contrary, moderate to high variations were observed in the number of specimens (for the polychaetes 68 to 90% at station D2 and 56 to 85% at station A2) (Tables 7a and 7b).

For stations K2 and K4 the wide variations suggest the existence of different biotopes i.e regarding species composition at K4, in April 79% were represented by polychaetes and 11% by molluscs, while in July only 63% of the species were polychaetes, 20% molluscs and echinoderms appeared for the first time with 2 representatives.

Table 5

Number of individuals per m² at each station in Faliro Bay.

	APRIL 1991						JULY 1991			
	A1	A2	K1	K2	K4	D2	A2'	D2'	K2'	K4'
POLYCHAETA										
<i>Aedicira mediterranea</i>	0	0	0	0	0	0	0	25	0	0
<i>Ampharete acutifrons</i>	0	10	0	0	20	20	0	5	0	15
<i>Amphictene capensis</i>	10	40	0	0	0	20	0	0	0	15
<i>Aonides oxycephala</i>	0	0	0	0	0	0	0	5	0	55
<i>Aricia foetida</i>	0	0	0	0	50	10	115	145	5	125
<i>Aricidea capensis</i>	0	0	0	0	0	0	5	0	0	0
<i>Aricidea curviseta</i>	0	0	0	20	50	20	20	55	15	30
<i>Aricidea fauveli</i>	30	130	10	130	30	90	55	210	0	35
<i>A. fragilis mediterranea</i>	0	0	0	30	120	90	0	35	0	25
<i>Aricidea sp.</i>	0	0	0	0	20	0	0	5	0	0
<i>Bhawania reyssi</i>	0	0	0	0	0	10	0	0	0	0
<i>Capitella capitata</i>	160	50	160	110	0	0	0	5	5	0
<i>Capitellidae sp.</i>	0	10	0	0	0	0	0	0	0	5
<i>Capitomastus minimus</i>	0	0	0	0	0	0	45	0	0	0
<i>Chaetozone setosa</i>	40	0	0	40	140	110	105	70	15	70
<i>Chone collaris</i>	0	0	0	0	0	0	0	0	0	10
<i>Chone filicaudata</i>	0	30	0	10	40	0	5	5	0	100
<i>Cirratulidae sp.</i>	0	0	0	10	0	0	0	0	0	0
<i>Cirrophorus branchiatus</i>	0	0	0	0	0	0	0	0	0	5
<i>Cossura coasta</i>	0	0	0	20	0	0	15	5	0	15
<i>Dasybranchus caducus</i>	0	0	0	0	0	0	0	45	0	45
<i>Euclymene Oerstedii</i>	0	0	0	0	10	0	0	15	0	225
<i>Eulalia viridis</i>	0	0	0	0	0	10	0	0	0	0
<i>Eunice vittata</i>	0	0	0	0	10	30	0	5	0	40
<i>Eunicidae sp.</i>	0	0	0	0	0	10	0	0	0	0
<i>Exogone gemmifera</i>	0	0	0	0	0	0	0	0	0	5
<i>Glycera convoluta</i>	10	0	0	20	30	0	0	0	0	5
<i>Glycera lapidum</i>	10	10	0	0	10	10	0	10	0	10
<i>Glycera Rouxii</i>	10	10	40	40	40	60	40	25	0	50
<i>Glycera unicornis</i>	20	0	0	10	10	0	0	10	15	20
<i>Goniada eremita</i>	0	0	0	0	20	0	0	0	0	10
<i>Heteromastus filiformis</i>	10	30	0	190	180	100	325	335	0	190
<i>Hyalinoecia brementi</i>	0	0	0	0	0	10	0	5	0	145
<i>Hydroides norvegica</i>	0	0	0	0	0	10	0	0	0	5
<i>Jasmineira caudata</i>	0	0	0	0	0	0	0	0	0	20
<i>Lagis koreni</i>	0	0	10	10	0	0	0	0	0	0
<i>Laonice cirrata</i>	0	20	0	50	200	220	5	50	0	35
<i>Leiochone clypeata</i>	0	0	0	0	0	50	0	130	0	25
<i>Lumbrineris latreilli</i>	0	40	0	80	650	890	80	780	15	815
<i>Magalia perarmata</i>	30	0	0	0	0	30	0	0	0	0
<i>Marphysa Bellii</i>	0	0	0	0	0	0	0	0	0	30
<i>Melinna palmata</i>	0	0	0	40	20	0	0	10	0	20
<i>Microspio mecznicowianus</i>	310	0	310	30	0	0	0	0	0	15
<i>Mysta picta</i>	0	0	0	0	10	0	0	0	0	0
<i>Nephtys hombergi</i>	0	0	0	0	0	0	0	5	0	0

Stations 3, 4 and 5 present considerably higher species richness ($S=157$, 167 and 191 respectively), moderate density values and an even distribution of the individuals among the different species ($J=0.724$, 0.778 and 0.839 respectively).

b) Faliro Bay

The values of the ecological parameters for the stations in the Bay of Faliro are shown at the end of Table 5 and presented graphically in Fig. 6. It is clear from both table 5 and Fig. 6 that the shallower stations A1 and K1, located closer to the mouth of the rivers Kifissos and Ilissos, are the most disturbed; values of community diversity (as low as 2.44 and 2.46 bits/unit), evenness of distribution (0.48 and 0.59) and species diversity (between 3.14 and 5.12), are comparable to those estimated for stations 1, 2 and 10 in the Saronikos proper.

3.4 Multivariate analysis

In order to test for variations among replicates, in the Saronikos Gulf, biotopes (groups of stations) were defined, on the basis of their faunal composition for each sample separately. Thus, 4 dendograms were constructed for the four sampling periods by using the raw data. The results are shown in Fig. 7 (a to d). In all seasons, there was a distinct separation of station 1 replicates at a very high similarity level (between 70 and 95%), while the replicates of the other stations were always grouped among themselves at lower similarity levels.

The replicates were subsequently lumped and a further dendrogram was created with seasons as replicates. The results were the same (see dendrogram of Fig. 8), proving that the seasonal changes were not big enough to obscure the situation. Three major groups were formed: stations 10 and 2 were clustered together, stations 5, 3 and 4 formed a second group and station 1 with a low similarity level (43%) was separated from the rest.

The same methods of Numerical Taxonomy were applied to the lumped data for Faliro bay, with seasons treated independently. The results are shown in Fig. 9a. Stations A1 and K1, situated closer to the mouth of the rivers Kifissos and Ilissos, at the isobath of 5 meters, were separated at a high similarity level. The remaining stations formed a distinct group at a lower similarity level (about 60%), except for station K2', sampled in July, which remained as a single site group.

A two-dimensional presentation of the results for Faliro Bay as derived with the M.D.S. technique is shown in Fig. 9b. The only clear grouping is that of stations A1 and K1.

4. DISCUSSION

According to Bellan *et al.* (1985) the organic enrichment of an ecosystem which is due to human activities, represents a factor of environmental degradation imposing changes to the communities' structure and species composition in proportion to its intensity. Josefson (1987) argues that the sedimentation of organic matter is the major cause of a common variability pattern and affects either one of the following: settlement,

somatic growth and survival on the bottom. Pearson and Rosenberg (1978) gathered quantitative data to describe the impact of pollution from urban wastes on benthic communities. Their study led to the construction of a model describing the change of some biological parameters (N, S, biomass and diversity index H') in relation to the gradual increase of organic matter.

In the present study based on the variation of the indices N, S, H, J and d, the faunistic data and the cluster analysis, the study area has been divided into zones corresponding to those of the model described by Bellan and Bellan-Santini (1972) and Pearson and Rosenberg (1978).

4.1 Saronikos Gulf

The lowest value of the indices S, and N was attained at station 1 which is closer to the outfall. The dominant species were Capitella capitata and Polydora antennata. In the summer, station 1 becomes azoic with none or only one species present (Corbula gibba) at very low densities (70 ind. m⁻²) (Table 4).

The species Capitella capitata is cited by many authors (Bellan and Bellan-Santini, 1972; Bellan, 1967; FAO/UNEP, 1986; Pearson and Rosenberg, 1978; Picard, 1965; Bellan *et al.*, 1985) as an indicator of domestic pollution. Gray (1979) suggested that the reason why this opportunistic species is dominant in organically enriched habitats is the lack of competitors, which are less tolerant of the prevailing conditions, combined with the high rate of reproduction of C. capitata which follows the r-adaptive strategy.

The polychaete Polydora antennata and the mollusc Corbula gibba, are also considered as pollution indicators (FAO/UNEP, 1986). Consequently, according to the model described by Bellan and Bellan-Santini (1972), station 1 corresponds to the polluted zone which becomes azoic during the summer. In the dendrogram of Fig. 8 the separation of the summer samples of station 1 from the rest of the samples of this station clearly reflects the azoic condition of this station during the summer. The same, is even more evident in the dendrograms of each season separately, based on the replicate samples (see Fig. 7a to d).

Stations 2 and 10 correspond to a transitional polluted zone characterized by a few opportunistic species which attain very high densities.

Chaetozone setosa which is extremely abundant in the summer samples of station 10 (reaching a density of 3060 ind. m⁻²), is considered as an indicator of natural or artificial perturbation of the environment and a species characteristic of transitional zones (Drago and Albertelli, 1976; Davies *et al.*, 1985; FAO/UNEP, 1986; Mair *et al.*, 1987). Among the abundant species at station 10 was the species Prionospio malmgreni which is also considered as an indicator of environmental instability (Pearson and Rosenberg, 1978; FAO/UNEP, 1986). Other species found in high densities such as Lumbrineris latreilli, Nereis caudata, and Prionospio pinnata are considered as characteristic of the transitional zone (Bellan, 1985) and as indicators of environmental instability and organic enrichment (Picard, 1965; Pearson and Rosenberg, 1978; FAO/UNEP, 1986).

The same is clearly reflected in Figs. 3 to 5. Namely by the low values of the diversity index ($H'=2.476$ at station 2 and $H'=3.046$ at station 10), the species diversity index ($d=6.479$ at station 2 and $d=9.399$ at station 10) and mostly by the evenness of the species distribution ($J=0.462$ at station 2 and $J=0.492$ at station 10).

Stations 3, 4 and 5 correspond to the transitional but less aggravated zone as indicated by the considerably higher species richness ($S=157$, 167 and 191 respectively), the moderate density values and the even distribution of the individuals among the different species ($J=0.724$, 0.778 and 0.839 respectively). It is noteworthy that the values of these parameters follow a gradient from station 3 (in the inner gulf) to station 5 (towards the outer gulf) that is as the distance from the pollution source increases (see Figs 3 to 5).

Among the abundant species at stations 3, 4 and 5 are Paralacydonia paradoxa and Nematonereis unicornis (at station 5) which characterise the transitional zone (Bellan, 1985) and the species C. setosa, L. latreilli, P. malmgreni, P. pinnata and T. heterochaeta.

In the dendrogram of Fig. 8 stations 3 and 4 form a group irrespectively of the seasonal variations observed, while the samples of station 5 are grouped into a separate group. This latter can be considered as a subgroup within the group of stations 5, 3 and 4 indicating that the community at station 5 further approaches the undisturbed condition.

Similar trends in the variation of the ecological parameters along a pollution gradient caused by domestic effluents are also recorded by other authors such as by Unsal (1988) for Golden Horn (Istanbul); by Aschan and Scullerud (1990) for the inner Oslofjord (Norway); by Kocatas (1981) for Izmir Bay and by Zarkanellas and Bogdanos (1977) and Zarkanellas (1979) for the Saronikos Gulf and the Bay of Elefsis.

4.2 In Faliro Bay

In stations A1 and K1 the values of community diversity (as low as 2.44 and 2.46 bits/unit) and evenness of species distribution (0.48 and 0.59) can be compared to those estimated for stations 1, 2 and 10 in the Saronikos Gulf. All other stations, with the exception of K4, presented intermediate values, but in all cases lower than those estimated for stations 3, 4 and 5 of the Saronikos Gulf. Station K4 (at 25 meters depth), had a fairly high number of species (39 and 87 seasonally) and an even distribution of individuals among species ($J=0.83$ and 0.79), resembling a situation close to normal. Species indicating disturbance due to organic pollution were present but not at critical levels.

In conclusion, the study area in Saronikos Gulf, could be divided into three zones: A heavily polluted zone around station 1, a transitional polluted zone (station 2, station 10) and a transitional but less aggravated zone (stations 3, 4 and 5). In Faliro Bay, a heavily polluted zone was evident around the shallower stations A1 and K1, situated near the mouth of the rivers Ilissos and Kifissos, and a transitional polluted zone, at stations A2, K2 and D2 at the isobath of 10 meters. The deeper station K4 presented a situation approaching normality.

This grouping was derived irrespective of the temporal variations observed. Classification applied separately on the data taken at each sampling period showed the same grouping. Thus, it is evident that the temporal variations in the faunal composition did not play a significant role in the distribution pattern.

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EVALUATION OF THE INFLUENCE OF SUSPENDED MATTER DUE TO CIVIL AND INDUSTRIAL DISCHARGES ON BENTHIC ROCKY COMMUNITIES

by

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ABSTRACT

The annual cycle of sedimentation along a vertical cliff on the Portofino Promontory (Ligurian Sea, Mediterranean Sea) was studied using sediment traps placed at 15, 20 and 25 m depth. The amounts of organic and inorganic matter, originating from the upper levels and due to biological activities and the erosion of the cliff, were compared with those collected in the water column close to the bottom, as suspended matter. An increase of the quantities of coarse matter was related to the rainfall and was higher in the superficial trap. On the contrary, fine sediments were mostly due to the local sea conditions, and increased from the superficial to the deepest trap. These data suggest that resuspension of fine sediments from the bottom may represent an important fraction of the settling matter along a cliff, mainly at lower levels.

1. INTRODUCTION

During 1990, the influence of detritus descending along a cliff near the Portofino Promontory and that of several water column parameters [particulate organic carbon (POC), particulate organic matter (POM), total organic matter (TOM), total suspended matter (TSM)], on the structure and functioning of the hard-bottom communities were studied, using the method proposed by Bavestrello et al. (1991).

Particular attention was given to the organic fraction present in the sediments and in the water column, which plays an important role in determining the structure and function of littoral communities. Along rocky-shores, only a small fraction of the primary production is consumed in situ, while a large quantity, firstly as coarse detritus and subsequently as particulate and dissolved matter, enters the trophic chains of the soft-bottom communities below (Odum and De la Cruz, 1967; Mann, 1972; Pelet, 1977; Evans et al. 1980; Hedges et al., 1988). Mainly during the winter, this sedimented matter is resuspended by wave action, thereby contributing to the organic fraction of the water column (Zunini-Sertorio and Fabiano, 1991).

Bavestrello et al. (1991), using a simple trap, were the first to evaluate the quantity of inorganic and organic matter settling on the rocky seabed at the Portofino Promontory. This quantity is strongly influenced by changes in meteorological conditions.

A year later, the relationship between this peculiar sedimenting process and the seasonal cycles of the TOM and TSM measured at the water-sediment interface and in the

water column, 1 m above, was studied, using the same type of trap.

2. MATERIALS AND METHODS

Three traps, of the type described by Bavestrello *et al.* (1991), were placed along a cliff near Punta del Faro (Portofino Promontory, Ligurian Sea). The first trap was placed at 15 m depth, inside a pre-coralligenous community, characterized by the green alga *Halimeda tuna*; the second, inside a *Dictyota dichotoma* "forest", at 20 m depth and the third, inside a *Corallium rubrum* facies, at 25 m depth. During the period January 1990 to January 1991, observations were made and samples were collected monthly by scuba diving. Each trap was filled with 50 cc of chloroform to prevent microbiological activity. Each sample was fixed in 4% formalin. The algal and animal fragments (serpulid tubes, barnacle exuvias, or other biological material) were removed from the samples, using a stereo-microscope and weighed separately. The sample was then dried at 60EC for 3 hours and weighed in two, fine ($\bar{O} > 0$) and coarse fractions ($\bar{O} < 0$, Wentworth scale). The organic matter content was expressed as the difference between the dry weight of the sediment and the residue left after combustion (ash free dry weight) at 550EC for 4 hours (Parker, 1983).

At each sampling station, 10 l of sea water were collected at the seabed level and 1 m above it, in order to analyze TSM, TOM and POM (as the sum of lipids, proteins, and carbohydrates). The water samples were filtered through a Whatman filter (GF/C 0.45 μm) for TSM, TOM and POM analysis and through a Millipore filter (0.8 μm), for pigment analysis.

Total suspended matter was analyzed by the gravimetric method after drying (3 hours at 60EC), while the organic matter was removed by drying at 550EC for 4 hours.

The analysis of particulate lipids was carried out following the methods described by Bligh and Dyer (1959) and Marsh and Weinstein (1966).

Particulate proteins were determined according to Lowry *et al.* (1951), using bovine serum albumin (Boeringer GMBH) as a standard. Particulate carbohydrates were assayed after Dubois *et al.* (1956).

Data on daily precipitation (measured in mm day^{-1}) and sea condition (measured as wave height in cm) were kindly provided by the Osservatorio Geofisico (University of Genoa).

Statistical analyses were carried out using the computer programme STAT.

3. RESULTS

The quantity of sedimented matter along the cliff of Punta del Faro (Table 1) shows a characteristic trend with high variations during the year at all depths considered (Fig. 1c). The average values were about 6 to 7 $\text{g m}^{-2} \text{day}^{-1}$. Higher values were recorded during the period between fall and spring, with a strong decrease during the summer. At the beginning of this

period, however, mainly at 20 and 25 m depth, a strong peak was observed, coinciding with heavy rainfall and rough seas (Fig. 1a,b).

Table 1

Amounts of coarse and fine sediments collected by the three traps during the sampling period.

Date	Coarse sediments g m ⁻² day ⁻¹			Fine sediments g m ⁻² day ⁻¹		
	Trap 1	Trap 2	Trap 3	Trap 1	Trap 2	Trap 3
09.02.90	0.90	1.00	1.12	2.56	8.18	7.28
16.03.90	1.06	0.83	0.78	5.55	8.33	10.60
05.05.90	2.42	1.67	1.66	1.15	1.67	2.72
01.06.90	0.23	0.00	0.00	2.08	0.98	1.99
14.06.90	0.00	2.54	0.00	2.58	7.77	8.51
10.07.90	0.00	1.60	1.33	1.68	1.31	1.67
02.08.90	1.17	2.12	1.17	0.96	1.17	1.00
23.08.90	2.80	4.29	2.06	2.42	0.91	2.37
06.09.90	2.03	1.75	1.26	1.79	0.48	2.05
19.09.90	2.33	5.24	4.91	3.50	0.88	4.51
07.11.90	10.14	2.30	8.74	5.63	0.86	8.85
03.12.90	6.20	5.83	7.76	4.16	6.66	10.03
01.02.91	2.84	6.94	2.68	6.55	9.64	6.79

Differences arose when studying the two granulometric fractions separately. The fine sediments ($\ddot{O} > 0$) collected by the superficial trap (15 m depth) (Fig. 2), were generally abundant in two distinct periods: February to the first half of March (about 6 g m⁻² day⁻¹) and October to January (about 6 to 7 g m⁻² day⁻¹); during these periods, the trend appeared to be linked to the sea condition. In the second and third traps (20 and 25 m depth) (Fig. 2), the fine sediments collected showed a strong correlation with the sea conditions with three peaks in February to first half of March, June, and October to January (about 8 to 10 g m⁻² day⁻¹).

The coarse sediments ($\ddot{O} < 0$) were mainly composed of animal debris (exuvias, serpulid tubes, shells) and inorganic matter. Most of this sedimentation was collected in the first trap (about 10 g m⁻² day⁻¹) in October (Fig. 3) and the annual trend is closely linked to the quantity of rainfall. The same was observed for the other two traps (Fig. 3), always well related to the pattern of rainfall. The algal debris (Table 1) was collected mainly at 20 m depth (Fig. 4), inside the *Dictyota dichotoma* "forest". In this trap, the quantity collected was three times more than in the other traps. All annual trends were related to the sea condition, particularly during the fall.

3.1 Water column parameters

Table 2 shows the TSM and TOM values measured in the water column. At the water-sediment interface, the TSM and the TOM trends were similar, with two peaks in June and September (Fig. 5). The same parameters, measured in the

Table 2

Amounts of total organic matter (TOM) and total suspended matter (TSM) measured in the water column at the water sediment interface and 1 m above the bottom during the sampling period.

	TOM interface	TOM 1m above	TSM interface	TSM 1m above
Date	mg l ⁻¹	mg l ⁻¹	mg l ⁻¹	mg l ⁻¹
09.02.90	2.56	1.66	9.85	7.55
16.03.90	2.63	2.81	8.26	9.12
05.05.90	3.52	2.8	9.78	6.83
01.06.90	4.4	1.98	12.94	5.14
14.06.90	3.19	2.15	11.39	9.77
10.07.90	2.73	3.09	9.93	10.84
02.08.90	4.05	3.41	13.06	9.74
23.08.90	3.85	3.17	16.04	10.93
06.09.90	6.1	3.87	21.40	7.90
19.09.90	5.18	5.66	18.50	17.69
07.11.90	4.45	4.35	14.13	15.00
03.12.90	4.07	4.05	13.34	10.38
01.02.91	4.78	3.7	17.70	10.88

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THE INFLUENCE OF POLLUTION ON THE PHYSIOLOGY OF CERTAIN
ZOOPLANKTONIC AND BENTHIC ORGANISMS FROM SARONIKOS GULF

by

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ABSTRACT

Digestive enzymatic activity and oxygen consumption rate of the pelagic copepod Acartia clausi and the benthic burrowing decapod Callianassa tyrrhena from the polluted-eutrophic Elefsis bay and the non-polluted-oligotrophic Vouliagmeni area, both situated in Saronikos Gulf, Aegean Sea, were compared. Out of 13 hydrolases of the whole body of A. clausi, 10 had a higher activity in the Elefsis population than in the one of Vouliagmeni. Only amylase, alkaline phosphatase and esterase lipase had a higher activity in the population of Vouliagmeni. On the contrary, in the case of C. tyrrhena, an "inhibitory" effect was observed, as the majority of the nineteen enzymes tested from the digestive gland, showed a lower activity in the polluted area. Only the activity of trypsin, β -galactosidase and α -glucosidase showed a clear increase in polluted waters. Generally speaking, more active enzymes were present in Callianassa than in Acartia. The respiration rate of Acartia was significantly lower in Elefsis, irrespective of season. A significant seasonal variation was observed which was greater in the Vouliagmeni population with a 50% decrease in the respiration rate between spring and summer. On the other hand Callianassa showed in general a very low respiration rate which, nevertheless, was affected by the locality and the size of the individuals. In polluted waters, mean respiration rate was lower than in the cleaner area (0.12 and $0.20 \mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$ respectively). The results obtained for Acartia agree with previous literature data and show the physiological characteristics of A. clausi in Elefsis bay that allow it to thrive in polluted-eutrophic waters. The low rates of respiration of Callianassa on the other hand, are a common feature of many callianassids so far studied, and have to be interpreted as a pre-adaptation of these burrowing species to low oxygen concentrations in the sediments.

1. INTRODUCTION

The present research project was carried out within the framework of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean (MED POL - Phase II).

The study concerns the effect of pollution on the physiology of certain zooplanktonic and benthic organisms (Acartia clausi and Callianassa tyrrhena respectively) which are present in coastal polluted and non-polluted areas of the Saronikos Gulf and in the Mediterranean sea in general.

The Saronikos Gulf (gulf of Athens), an extension of the Southern Aegean sea, is an area of particular socio-economic importance. The object of the study was to complete existing data concerning the effect of pollution on organisms, planktonic and benthic, of the Saronikos Gulf and the marine coastal areas in general. The effects of heavy metals and hydrocarbons on the ecophysiology of different marine organisms (A. clausi) have been studied taking into account certain environmental factors such as, food, temperature, salinity, light etc (Moraitou-Apostolopoulou and Verriopoulos, 1976; 1978; 1981a; 1981b; Moraitou-Apostolopoulou et al., 1979; Verriopoulos and Moraitou-Apostolopoulou, 1981; 1982; Verriopoulos et al., 1989); the enzymatic activity of planktonic organisms in relation to pollution was also studied (Kerambrun, 1983; 1984; 1988; Rivière and Kerambrun, 1983; Kerambrun and Guerin, 1984; Gaudy et al., 1991). For C. tyrrhena mercury and cadmium bioaccumulation and their effects on soluble peptides, proteins and enzymes were studied (Thaker and Haritos, 1989a; 1989b).

In order to obtain information about the physiological situation of the organisms, we examined their metabolism (respiration rate) and enzymatic activity.

A. clausi. A very common planktonic form, with a very wide horizontal distribution. Because of its local abundance, its biological cycle has been studied by many researchers. The number of annual generations is variable and seems to depend primarily on latitude. Eight generations each year have been estimated in the Black sea (Porumb, 1968), four in Long Island Sound (Conover, 1956), five in the Plymouth area (Digby, 1950) and six in the gulf of Marseille (Gaudy, 1972).

A. clausi is found in great numbers in the coastal areas of the Aegean sea during winter. In the Saronikos Gulf A. clausi constitutes a basic element of the planktonic community as it is true also for other parts of the world (Moraitou-Apostolopoulou, 1974; 1977; Ueda, 1978; Scotto di Carlo and Ianora, 1983; Pagano and Saint-Jean, 1983; Gaudy, 1984).

C. tyrrhena. The ghost shrimp has an eastern Atlantic-Mediterranean distribution. Its Atlantic range extends from the English Channel to the coasts of Mauritania (Saint Laurent and Bozic, 1976; Saint Laurent and Le Loeuff, 1979). In Greece, it has been reported from many mainland and island coasts (Thessalou-Legaki, 1986). The species lives in very shallow waters up to a few meters (Picard, 1957; Manning, 1975; Saint Laurent and Bozic, 1976; Pastore, 1976; Saint Laurent and Manning, 1982; Garcia Raso, 1983). It forms dense populations on sand flats (Le Gall, 1969; Ott et al., 1976). The impact of C. tyrrhena on redox potential and nutrient cycling of the sediment has been documented by Ott et al. (1976). The role of callianassids in shallow-water ecology has recently received increasing attention. The biology of the species from Greek waters has been the subject of some recent studies (Thessalou-Legaki, 1988; 1990).

2. MATERIALS AND METHODS

2.1 Sampling sites

Two areas in the Saronikos Gulf were chosen (Fig. 1):

The evaluation of dry weight was made after the organisms had been kept at 70EC for 24 hours (zooplankton) and 72 hours (benthic animals in some cases).

2.3 Respiration rate

The oxygen consumption measurements were carried out in completely dark, constant temperature rooms, where the temperature was similar to that of the sampling area. The animals were placed in 500 ml Erlenmeyer flasks filled with oxygen-saturated water collected from the "non polluted" area. This natural water was either filtered through a Whatman glass microfibre (GF/C) filter (for all Callianassa measurements and Acartia without food) or used as it was collected (for Acartia with food). The estimation of oxygen consumption was made with an oxymeter (YSI model 51B) after 24 hours (Omori and Ikeda, 1984).

The weight specific respiration rate (R) was calculated using the following equation:

$$R = (C_{ox} - E_{ox}) 1000V/t/dw$$

where:

C_{ox} and E_{ox} is the dissolved oxygen content ($ml O_2 l^{-1}$) in the control and experimental flasks respectively; V is the volume of the medium (l); t, the incubation time (h) and dw the dry weight of animals (mg).

R is expressed as weight-specific respiration rate ($\mu l O_2 mg dw^{-1} h^{-1}$) from here on, referred to as respiration rate for both species.

For Callianassa, in most of the cases, the dry weight of the animals was calculated from length-weight relationships that already existed for the species from the same season and for males, ovigerous and non-ovigerous females separately (Thessalou-Legaki, 1988).

Additionally, the total respiration rate of an individual ($\mu l O_2 animal^{-1} h^{-1}$) was calculated for Callianassa.

2.4 Enzymatic activities

Initially, electrophoresis was the only technique foreseen for this study. Nevertheless, in order to minimise the fragmented character of the results obtained through electrophoresis, several tests were performed using the API-ZYM (Bio Merieux, France) technique.

2.4.1 Preparation of extracts

The preparation of the extracts destined for electrophoresis was carried out through stirring of the samples in a pH 6.8 buffer solution containing 10% glycerol and bromophenol blue. The extracts destined for API-ZYM were prepared in a non buffered solution of NaCl (0.75%), after centrifugation at 15000 g for 10 min.

The A. clausi samples (per 100 individuals) were also homogenised in a 75 µl buffer solution. Total plankton samples were homogenized in NaCl solution (0.75%). After centrifugation, an aliquot was mixed with the pH 6.8 buffer solution containing glycerol and bromophenol blue in order to obtain extracts of concentration similar to the A. clausi extracts. The remaining non buffered extract was used in the API-ZYM.

As far as the decapods are concerned, various extracts were prepared from the digestive gland, the muscle of the P₁ pincer and the abdomen. Samples of 20-40 mg were homogenised in a NaCl solution (0.75%) using 60 µl for about 10 mg. After centrifugation, the aliquot destined for electrophoresis was mixed with the pH 6.8 buffer solution containing glycerol and bromophenol blue. The rest, non buffered, was used in the API-ZYM.

2.4.2 Electrophoresis

Electrophoresis was realised on vertical gel with 7.5% polyacrylamide, with a pH 8.5 Tris-glycine buffer in the gel and pH 8.1 in the electrode basins.

Esterases were revealed in 50 ml of phosphate buffer 0.1M (pH 6.5) containing 1 ml of acetone solution (2.0%) of á-naphthyl acetate and 50 mg of Fast Red TR after pre-incubation for 30 min in a solution of boric acid (0.5 M at 4EC). Alkaline phosphatase was revealed through gel incubation in a 50 ml Tris-HCL buffer 0.05M pH 8.8 containing 50 mg of á-naphthyl phosphate, 50 mg of Fast blue BB, 60 mg MgCl₂ and 5 mg of PMS for 50 ml. For leucine aminopeptidase, incubation took place in a Tris-maleate/NaOH buffer 0.05M, pH 6, containing 20 mg of L-leucyl-b-naphthylamide HCl, 15mg of Black K salt and 50mg of MgCl₂ for 50 ml. á-amylase was revealed through an iodine solution after incubation in a phosphate buffer 0.05M, pH 6.9, containing 1% amidon. Malate dehydrogenase (MDH) was revealed by incubation in a Tris-HCl buffer 0.05M, pH 8.25 containing 160 mg of sodium malate, 30 mg of -NAD, 15 mg of NBT and 15 mg of MTT, 60 mg of MgCl₂ and 5 mg of PMS for 50 ml. For the malic enzyme (ME), incubation was realised in a Tris-HCl buffer 0.25M, pH 8.0, containing 160 mg of sodium malate, 15 mg of NADH, 15 mg of NBT, 10 mg of MTT, 60 mg of MgCl₂ and 5 mg of PMS for 50 ml.

2.4.3 API-ZYM

The API-ZYM system (Bio Merieux, France) which was used, is a semi-quantitative research micromethod for enzymatic activities which allows the simultaneous testing of 19 enzymatic activities from a small sample (Monget, 1978): alkaline phosphatase, esterase (C₄), esterase lipase (C₈), lipase (C₁₄), leucine aminopeptidase, valine aminopeptidase, cysteine aminopeptidase, trypsin, á-chymotrypsine, acid phosphatase, phosphoamidase, á and â-galactosidases, â-glucuronidase, á-glucosidase, â-glucosidase, N-acetyl-â-glucosaminidase, á-mannosidase and á-fucosidase.

2.5 Statistical analysis

The statistical treatment of the results both on A. clausi and C. tyrrhena was based mainly on a Multifactor Analysis of Variance taking into account the following factors:

- a) Acartia diet (with-without food); Callianassa (without food)
- b) season (spring -summer) Acartia and Callianassa,
- c) location (Elefsis - Vouliagmeni) Acartia and Callianassa,

- d) sex (females) Acartia; (males - non-ovigerous females - ovigerous females) Callianassa and
- e) dry weight (in mg) Callianassa

In both species the factors affecting the respiratory activity were:

<u>Factor</u>	<u>level</u>
season	1: spring 2: summer
location	1: Elefsis 2: Vouliagmeni

In the case of Acartia, diet was examined:

diet	1: with food 2: without food
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In the case of Callianassa the animals were treated individually and thus two more factors were added:

sex	1: males 2: non-ovigerous females 3: ovigerous females
DWcode (dry weight in mg)	1: 1 - 299 2: 300 - 599 3: 600 - 899 4: 900 - 1199

3. RESULTS

3.1 Electrophoresis

3.1.1 Acartia clausi

The comparison of 2 samples collected on 4 March 1991 (with 200 and 100 ind.) originating from the bay of Elefsis and of 2 samples collected on 27 March 1991 (with 100 ind. each) originating from Fleves-Vouliagmeni revealed certain activity differences between localities.

In general, the esterase activities appeared to be more intense in the Elefsis samples (Fig. 2). In the Fleves samples, certain fractions were not detectable. As far as amylase activity is concerned, it is manifested by a clear fraction which is more intense in the Fleves-Vouliagmeni samples while the sample collected on 4 March from Elefsis shows the faintest activity. Leucine amino-peptidase gives a fraction which seems to be unique and of faint activity (Fig. 3).

Alkaline phosphatases were only revealed in the sample collected on 27 March originating from Fleves-Vouliagmeni. No activity was manifested in the samples collected on 4 March originating from Elefsis. Finally, no activity was evident for MDH (Malate dehydrogenase) and EM (Malic enzyme).

Table 1

Respiration rate of *Acartia clausi*.

Level		Count	Average	Std. Error (internal)
ACARTIA food (1=with, 2=without)				
1		14	3.8600000	.8807714
2		14	5.3264286	.8918536
ACARTIA season (1=spring, 2=summer)				
1		15	5.9080000	.7060225
2		13	3.0761538	.9489431
ACARTIA locality (1=Elefsis bay, 2=Vouliagmeni-Fleves)				
1		13	3.4607692	.6905725
2		15	5.5746667	.9648315
ACARTIA food by ACARTIA season				
1	1	7	5.3985714	1.4116377
1	2	7	2.3214286	.7614661
2	1	8	6.3537500	.5602580
2	2	6	3.9566667	1.8928368
Total		28	4.5932143	.5607908
ACARTIA food by ACARTIA locality				
1	1	6	3.2133333	.5672252
1	2	8	4.3450000	1.5067989
2	1	7	3.6728571	1.2350086
2	2	7	6.9800000	1.0050207
ACARTIA season by ACARTIA locality				
1	1	7	4.2828571	.9892727
1	2	8	7.3300000	.7185079
2	1	6	2.5016667	.8763767
2	2	7	3.5685714	1.6457614
Total		28	4.5932143	.5607908

ovigerous females; 3=ovigerous females), season (1=spring; 2=summer), locality (1=Elefsis bay; 2=Vouliagmeni), dry weight (1=1-299 mg; 2=300-599 mg; 3=600-899 mg; 4=900-1199 mg) and the interactions sex-season, sex-locality and locality-dry weight.

Statistically significant dry weight effects were observed for the total respiration rate (F-ratio = 30.105; Sig. level = 0.0000). The total respiration rate increases with dry weight (Fig. 9).

A statistically significant seasonal variation is observed for the total respiration rate (F-ratio = 6.638; Sig. level = 0.0133). The total respiration rate of the individuals collected in summer is about 1.3 times lower than those collected in spring (Fig. 10).

Table 2

Total respiration rate ($\mu\text{l O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) of *Callianassa tyrrhena*.

Level		Count	Average	Std. Error (internal)
CALLIAN.sex (1=males; 2=non-ovigerous females; 3=ovigerous females)				
1		23	56.46804	6.205513
2		21	63.57619	5.265705
3		18	73.32722	5.692896
CALLIAN.season (1=spring; 2=summer)				
1		34	70.50779	5.194517
2		28	55.58893	3.677972
CALLIAN.locality (1=Elefsis bay; 2=Vouliagmeni)				
1		24	66.79021	5.868341
2		38	61.86289	4.178851
CALLIAN.DWcode (1=1-299 mg; 2=300-599 mg; 3=600-899 mg; 4=900-1199 mg)				
1		23	39.53130	2.957837
2		9	60.84000	3.506722
3		24	78.26813	4.163881
4		6	103.09000	6.124550
CALLIAN.sex by CALLIAN.season				
1	1	13	64.93500	9.654342
1	2	10	45.46100	5.579276
2	1	15	65.45933	6.324942
2	2	6	58.86833	10.096876
3	1	6	95.20333	9.812355
3	2	12	62.38917	4.540746
CALLIAN.sex by CALLIAN.locality				
1	1	7	35.73143	6.652807
1	2	16	65.54031	7.444065
2	1	6	73.65583	6.317439
2	2	15	59.54433	6.759430
3	1	11	82.81000	7.443678
3	2	7	58.42571	5.517757
CALLIAN.season by CALLIAN.locality				
1	1	10	75.28950	11.321774
1	2	24	68.51542	5.775791
2	1	14	60.71929	5.852896
2	2	14	50.45857	4.229110
CALLIAN.locality by CALLIAN.DWcode				
1	1	6	32.81417	6.907728
1	2	3	51.82667	7.192159
1	3	11	75.43545	4.203576
1	4	4	105.20250	9.136817
2	1	17	41.90206	3.109003
2	2	6	65.34667	2.642268
2	3	13	80.66500	6.917030
2	4	2	98.86500	5.915000
Total		62	63.77024	1.985374

Table 3

Respiration rate ($\mu\text{l O}_2 \text{ mg dw}^{-1} \text{ h}^{-1}$) of *Callinassa tyrrhena*.

Level		Count	Average	Std. Error (internal)
CALLIAN.sex (1=males, 2=non-ovigerous females; 3=ovigerous females)				
1		23	.1888301	.0277664
2		21	.1911253	.0316330
3		18	.1303968	.0144408
CALLIAN.season (1=spring, 2=summer)				
1		34	.1761576	.0196673
2		28	.1683754	.0253180
CALLIAN.locality (1=Elefsis bay; 2=Vouliagmeni)				
1		24	.1233174	.0108298
2		38	.2037960	.0232420
CALLIAN.DWcode (1=1-299 mg; 2=300-599 mg; 3=600-899 mg; 4=900-1199 mg)				
1		23	.2826089	.0299167
2		9	.1206492	.0051466
3		24	.1059705	.0057412
4		6	.0957879	.0080813
CALLIAN.sex by CALLIAN.season				
1	1	13	.1590945	.0215267
1	2	10	.2274865	.0568998
2	1	15	.2170500	.0382076
2	2	6	.1263137	.0511283
3	1	6	.1108965	.0048955
3	2	12	.1401469	.0212615
CALLIAN.sex by CALLIAN.locality				
1	1	7	.1435986	.0244023
1	2	16	.2086189	.0378877
2	1	6	.1315466	.0305224
2	2	15	.2149568	.0415478
3	1	11	.1059226	.0068549
3	2	7	.1688562	.0315099
CALLIAN.season by CALLIAN.locality				
1	1	10	.1507416	.0216409
1	2	24	.1867476	.0263303
2	1	14	.1037288	.0073000
2	2	14	.2330220	.0443438
CALLIAN.locality by CALLIAN.DWcode				
1	1	6	.1788826	.0316733
1	2	3	.1290240	.0098016
1	3	11	.1006485	.0070041
1	4	4	.0980294	.0123803
2	1	17	.3192182	.0351175
2	2	6	.1164618	.0058168
2	3	13	.1104738	.0088517
2	4	2	.0913050	.0054626
Total		62	.1726430	.0110124

Table 4

Summary of respiration rate results.

1. ACARTIA	
Respiration rate	
significant	not significant
season (locality)	food
all interactions not significant	
2. CALLIANASSA	
Total respiration rate	
significant	not significant
dry weight season	sex locality
all interactions not significant	
Respiration rate Total number of individuals	
dry weight locality	sex season
all interactions not significant	
Individuals of >300 mg dry weight	
season	sex locality
Interactions: sex-season sex-locality	
Individuals of <300 mg dry weight	
(locality)	sex season

Differences in temperature tolerance have been observed between the three annual generations. At the upper temperature limit, Acartia of the cold period were less resistant than Acartia of the warm and intermediate periods. The specimens from the polluted area proved to be more resistant both to the upper and to the lower temperature limits (Moraitou-Apostolopoulou and Verriopoulos, 1981a).

The pollution adapted Acartia population survived longer, under laboratory controlled conditions, than the non-adapted population (Moraitou-Apostolopoulou and Verriopoulos, 1981b).

The results of the respiration rate of Callianassa showed that in all cases the sex or the reproductive phase (ovigerous or non-ovigerous females) do not have any direct effect on the respiration rate (both total and weight-specific).

Season and locality significantly affected both the total and weight-specific respiration rate. By splitting the total populations into large and small individuals, it has been shown that the two groups are differently affected: season affects the respiration rates in larger animals, while smaller ones seem to be more sensitive to eutrophication.

In general, the low rates of respiration of C. tyrrhena, obtained from the present study are well in agreement with the data from other studies of thalassinids from coastal or intertidal sediments, such as C. californiensis (Thompson and Pritchard, 1969; Miller et al., 1976; Torres et al., 1977) and C. jamaicense (Felder, 1979). These species show respiration rates that are among the lowest observed for decapods. This fact is interpreted as an adaptation which allows them to maintain aerobic respiration over a wide range of ambient PO_2 . They are also highly tolerant of severe hypoxic and even anoxic conditions. From this point of view, a further study on the respiratory pattern of the species might provide evidence on the tolerance range and clarify the role of the environmental factors related to eutrophication. Although the species might be considered as highly tolerant, it exhibited a considerable sensitivity to the environmental factors tested.

Cadmium accumulation was found to cause qualitative and quantitative changes of soluble protein components in the hepatopancreas of the shrimp C. tyrrhena. Additionally, the molecular targets of cadmium appear to be versatile rather than limited (e.g. to metal-binding proteins). Further investigation in this direction might prove useful in the development of much needed sensitive and reliable bioassays for metal toxicity based on protein expression (Thaker and Haritos, 1989a).

Mercury accumulation was also found to cause significant qualitative and quantitative changes to the levels of soluble protein components in the hepatopancreas of the shrimp C. tyrrhena (Thaker and Haritos, 1989b).

It is also notable that the respiration rates of Acartia and Callianassa show some prominent common features:

- In both species, spring respiration rate means are higher than those of the summer
- In both species, the individuals from the eutrophic region show lower respiration rates.

In some cases of the present study, more evidence on the effect of pollution could have been provided if the degrees of freedom were higher. More measurements are needed in order to establish the complete pattern of respiratory activity of these two species and the relative importance of eutrophication, compared to other factors.

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