FOOD AND AGRICULTURE ORGANIZATION OF THE
UNITED NATIONS

UNITED NATIONS ENVIRONMENT PROGRAMME

MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 3 - Sampling and Analyses of Biological Material (Guidelines for the FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean)

by

M. Bernhard*
(Consultant)
Fishery Resources and Environment Division
Food and Agriculture Organization
of the United Nations
Rome, Italy

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PREPARATION OF THIS MANUAL

This Manual was prepared as part of a cooperative project of the United Nations Environment Programme entitled:

FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean

with the Food and Agriculture Organization of the United Nations as cooperating agency.

*present address of the author:
Divisione per la Protezione Ambientale
Comitato Nazionale per l'Energia Nucleare
19030 Fiascherino-Lerici, Italia

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FAO manuals. Methodology. Marine pollution. Monitoring. Sampling. Analytical techniques. Heavy metals. Organochlorines.

PREFACE

Under the Mediterranean Action Plan, developed by the United Nations Environment Programme and endorsed by the Intergovernmental Meeting on the Protection of the Mediterranean (Barcelona, 28 January - 4 February 1975), the Co-ordinated Mediterranean Pollution Monitoring and Research Programme was established. As part of this programme, an FAO(GFCM)/UNEP Joint Co-ordinated Project on Pollution in the Mediterranean was initiated. The project, among other subjects, aims at organizing baseline studies and monitoring of metals, particularly mercury, and of DDT, PCBs and other chlorinated hydrocarbons, in marine organisms.

The Operational Document, which serves as the programmatic basis for the collaboration of Mediterranean laboratories on baseline studies and monitoring of these pollutants, was developed at the Expert Consultation on the Joint Co-ordinated Project on Pollution in the Mediterranean convened by FAO(GFCM) in Rome, 23-27 June 1975. This document specifies the organisms and pollutants to be monitored and outlines the general methodology which should be followed.

At the request of the Expert Consultation, this Manual has been prepared to elaborate on the sampling and analytical procedures accepted in the Operational Document. Its aim is to give assistance to laboratories participating in the project in serving as a guide for sampling, sample treatment and analytical procedures agreed to by the Expert Consultation. Thus, it is envisaged to increase the comparability of the results and data obtained by the different laboratories.

Since it is expected that during the first months of use of this Manual comments and proposals for improvement will be received, it is envisaged to prepare a revised version of this Manual based on these comments and on advice of a second Expert Consultation to be convened at a later stage.

Final editing of the Manual was carried out by the staff of the Fishery Resources and Environment Division of FAO, particularly Mr. A. Wenblad. The views expressed in the Manual are those of the author and do not necessarily represent the views of either FAO or UNEP.

Figures 1 - 18 have been reprinted from: FAO Species Identification Sheets for Fisheries Purposes, Mediterranean and Black Sea (Fischer, 1973).

C O N T E N T S

1. INTRODUCTION

This Manual should be used in connection with the Report of the FAO(GFCM)/UNEP Expert Consultation on the Joint Co-ordinated Project on Pollution in the Mediterranean (FAO/UNEP, 1975), which serves as the Operational Document for this Project.

At this consultation a distinction was made between two priorities, pollutants of first priority are to be analysed in the organisms of first priority by all participants. The analyses of additional pollutants of secondary priority is encouraged and should be carried out whenever possible.

Table I gives a summary of the metals, chlorinated hydrocarbons (Chl-HC) and organisms mentioned in the operational document with their respective priorities. As a minimum requirement two metals and three chlorinated hydrocarbons and their metabolites should be determined in the Mediterranean mussel Mytilus galloprovincialis, and in the striped mullet Mullus barbatus. Since tuna is reported so far, as the fish which has the highest Hg concentration in the Mediterranean (Cumont et al., 1973), Hg and Cd will also be analysed in tuna. The deep water pink shrimp Parapenaeus longirostris was chosen as a third species for the analyses of Chl-HCs.

The organisms are to be collected at least four times a year, i.e. in September, December, March and June.

All institutes taking part in the projects will participate in an intercalibration exercise in order to facilitate a comparison of the analytical data obtained. Although for obvious reasons no standard analytical techniques are recommended in the operational document, atomic absorption spectrophotometry (AAS) and gas chromatography (GLC) will be used as reference methods for determination of metals and chlorinated hydrocarbons (Chl-HC) respectively.

The proposed "minimum programme" of the pilot projects was purposely limited to composite samples of a minimum number of species and pollutants so that as many research centres as possible could participate. It must, however, be viewed as a first approach to a comprehensive study of the pollution of the Mediterranean. Whenever possible the concentration in single specimen samples should also be determined, parallel to the composite samples, so that the necessary data for a statistical evaluation of the variability are available. Only in this way will it be possible to establish pollution trends. It would also be of great interest, if the participating centres could extend their investigation beyond the minimum programme outlined in the operational document and determine the concentrations of these pollutants in sea water and sediments and in representative species at the various levels in the food chain.

The following outline is written under the assumption that either a biologist or a chemist will be responsible for carrying out the programme. Either should, however, ask for support from biologists or chemists respectively, in order to guarantee that meaningful data are produced. Since contamination of the samples will present one of the major problems in the analyses it is suggested that persons responsible for the analytical determinations also participate actively in the planning of the sampling procedure so that contamination of the samples during sampling and sample preparation are minimized. Often valuable time is spent in chemical analyses of contaminated samples, or those otherwise unsuitable for analysis, because insufficient care and consideration has been given to avoid contamination during sampling, sample storage and sample preparation.

2. IDENTIFICATION OF ORGANISMS TO BE MONITORED

An accurate identification of the species sampled for the chemical analyses is necessary since different species of the same taxonomic groups may accumulate elements to various degrees. Thus, a concentration which can be considered normal for certain organisms may indicate pollution in others. Even for the relatively inexperienced person, the identification of the organisms of first priority (Mytilus, Mullus, Thunnus, Xiphias, Parapenaeus and

Table I

Priorities of elements and substances to be monitored

Elements of 1st priority	:	total-Hg, Cd
2nd priority	:	As, Cu, Pb, Mn, Se, Zn and methyl-Hg
Organisms of 1st priority	:	Mytilus galloprovincialis Mullus barbatus Thunnus thynnus (Xiphias gladius)
Chlorinated hydrocarbons of 1st priority	:	DDT, Dieldrin, PCBs and their metabolites
2nd priority	:	other persistent organic substances
Organisms of 1st priority	:	Mytilus galloprovincialis Parapenaeus longirostris (Carcinus mediterraneus) Mullus barbatus
Other organisms	:	Patella sp. Merluccius merluccius Trachurus trachurus Sardina pilchardus Sarda sarda Macrophytes Phyto/zooplankton Organisms living in sediments

Carcinus ssp.) should not be too difficult with the aid of the "FAO species identification sheets" (Fischer, 1973). All species, with the exception of Patella, are described in the FAO species identification sheets in Figs. 1-18. A key for the identification of Patella is given in Fig. 19.

The identification sheet gives a drawing of the organism; its distinctive characters and diagnosis, advice on distinction from similar species, common and maximum length range, geographical distribution and behaviour, present fishing grounds, production figures and the main fishing gear used. It also gives the common names used in most Mediterranean countries which may be helpful when dealing with commercial fishermen. It should, however, be remembered that only the scientific name should be used when making reference to the species in these pilot projects, since in different areas the same common names are often used for quite different species.

If additional information is needed, several faunas and floras can be consulted. For example Bini's (1965) Catalogue of names of fishes, molluscs and crustaceans of commercial importance in the Mediterranean (in English). Bini's (1967) Atlas of fishes from the Italian coasts and Luther and Fiedler's (1965) Guide to the marine coastal fauna of the Mediterranean, are available in Italian, the latter being a translation of a similar text in German (Luther and Fiedler, 1961). Palombi and Santarelli (1961) have written a book in Italian on the commercial marine animals. In the Fauna d'Italia, (in Italian) Tortonese (1963, 1965) has treated Echinodermata, Leptocardia, Cyclostomata, Selachii, etc. Riedl (1963) has published a book on the fauna and flora of the Adriatic Sea, in German. In French the following publications can be mentioned: a translation of Luther and Fiedler's book (1965a); Dieuzeide et al., (1953-55) - Catalogue des poissons des côtes algériennes and Collignon and Alonole (1972, 1973) - Catalogue raissone des poissons des mers marocains. In addition, the Faune iberia (Lozano Rey, 1947, 1949, 1952) and Solyan's (1965) Marine fishes of the Adriatic may be mentioned (in Croatian).

2.1 Precautions recommended for a correct identification

Although no great difficulties are anticipated in the identification of the species, also by the inexperienced person, it would be advisable if some specimens of each species collected are preserved in formaline, alcohol or deep frozen so that a later identification check by an expert can be carried out. A formaline preservation solution is easily prepared by adding 100 ml of analytical grade formaline (45% formaldehyde) to one litre of distilled water. The final solution should contain about 4% formaldehyde. The 75% alcoholic preservation solution should be prepared from analytical grade ethanol diluted with distilled water. If an alkaline formaline solution is required the 4% formaldehyde solution is saturated with borax by adding about 7 g borax for 100 ml, agitating the solution until all borax is dissolved. The pH determined with a pH meter should be between 8.0 and 8.5.

In the following, some indications are given to assist in the identification of the species mentioned in the expert consultation. In general once a certain familiarity has been obtained the different species will be recognized at first sight and the characteristics mentioned below are no longer needed to distinguish the species. An experienced person will recognise the different species by other more general characteristics such as the shape of the body, the fins, etc. which are not so easily described and quantified but nevertheless allow an easy differentiation.

2.2 Identification of the species of first priority

The identification of Mytilus galloprovincialis is easy for the layman (Fig.4). Although in the FAO identification sheets M. galloprovincialis is distinguished from M. edulis and Perna perna, it is not quite clear if M. galloprovincialis is really a separate species or only a variation of M. edulis (Riedl, 1963). P. perna or Mytilus perna occurs only along the coast of Algeria and northern Tunisia and thus cannot be confused with M. galloprovincialis in other areas.

Carcinus mediterraneus also has a very typical and characteristic shape (Fig. 5) which makes it difficult to confuse it with other crabs. The nearest species C. maenas is, however, very similar. It can be distinguished by the minor differences mentioned in the FAO identification sheet, although some doubt may exist as to whether the two species are really independent or only variations.

The identification of Parapenaeus longirostris requires a little more attention. This shrimp belongs to the Penaeidae. This taxonomic group can easily be distinguished from other shrimps since the first three pairs of thoracic limbs or pereiopods terminate in pincers (see Fig. 2 for morphological terms). In order not to get confused with other appendages it is easier to count the thoracic limbs from the end of the carapace or cephalothorax. The shape of the rostrum and the dorsal tooth helps to distinguish P. longirostris from other Penaeidae (see Fig. 6-8).

The genus Mullus can easily be identified by its characteristic reddish-pink colour of the back and the sides, one pair of long barbels under the chin (other species have one or four barbels) and a toothless upper jaw. The first fin has 8 or 9 spines and the second fin which is distinct from the first, one spine and eight soft rays. The characteristics necessary to distinguish M. barbatus from the other similar species are listed in the identification sheets, Fig. 9-11.

_____ _____ belongs to the Scombridae which are easily recognised by the typical finlets between the 2nd dorsal and the anal fin and the tail (Fig. 12). T. thynnus has a very typical shape and appearance which easily distinguishes it from other fishes. Its main differences from other similar Scombridae are listed under "Distinction from most similar species occurring in the area" in Fig. 12.

The swordfish X. gladius (Fig. 13) only resembles the genus Tetrapturus. The swordfish has, however, no pelvic fins and only one central keel on each side of the caudal peduncle instead of the two keels on either side in the Tetrapturus. The swordfish's sword is also much longer and more flattened than the one of the genus Tetrapturus.

2.3 Identification of other species

Trachurus trachurus has two dorsal fins, the first with spines connected by a membrane and higher than the second (Fig. 14). Most characteristic in all Carangidae are the two spines connected by a membrane in front of the anal fin. Trachurus differs from other Carangidae by a very marked and conspicuous row of large bony scutes along the lateral line showing a slope above the anal spines. The features to distinguish T. trachurus from the other two species are given in Fig. 14.

The sardines of the family Clupeidae can easily be distinguished from the anchovy Engraulidae by the shape of the mouth and slender form of the body (Fig. 15). In the anchovy, e.g. Engraulis encrasicolus the mouth extends well past the eye, its upper jaw is much longer than that of the sardines, so that the mouth opens below the head (subterminal mouth), while in the sardines the lower jaw is brought forward so that the mouth opens upwards (terminal mouth) (Fig. 15). The different species of sardines are not so easily distinguished. Sardina pilchardus differs from the other sardines by pronounced radiating ridges on the gill cover. In Fig. 15 the other characteristics necessary to distinguish S. pilchardus from other sardines are listed.

Merluccius merluccius is characterized by its two dorsal fins, the first higher than the second (Fig. 16). The second dorsal fin and the single anal fin are long. It has no barbel under the chin. The fishes most similar to Merluccius are two species of Phycis, two of Molva and several Gaidropsarus species. The two Phycis (Fig. 17) species can easily be distinguished from the Merluccius by their peculiar pelvic fins and the presence of a single barbel only under the chin. The two Molva species, M. molva and M. elongata possess a very elongated and narrow body and very thin ventral fins and a small barbel only under the chin.

The Sarda sarda is a scombrid (Fig. 18) and hence possesses the typical finlets between dorsal and anal fins and the tail fins. Indications to differentiate it from T. thynnus are given in Fig. 12. It can also be easily recognized by its characteristic dark oblique stripes.

The FAO identification sheets do not contain a description of the limpet Patella, which is a gastropod and thus has only one shell in the form of a cup or short cone. In the Mediterranean there are several species of Patella whose characteristics and distinction from other similar species are described in Fig. 19.

2.4 Identification of phyto- and zooplankton

In the expert consultation the monitoring of plankton, i.e. phyto- and zooplankton caught with plankton nets was discussed. Since plankton samples vary widely in species composition, analyses of metals and chlorinated hydrocarbons in plankton samples without an indication of the species composition is of little use. It is, therefore, absolutely necessary to preserve, for taxonomic analysis, an aliquote which has been drawn from a thoroughly mixed sample in order to be representative. These analyses can only be carried out by persons who are experienced, not only in the taxonomy of phyto- and zooplankton, but also are familiar with quantitative aspects of plankton sampling and analyses, (Bernhard et al., 1973). Phyto- and zooplankton can be identified with the help of Tregouboff and Rose's Manuel de Planctonologie Méditerranéene (1957) and Massuti and Margalef's Introducción al estudio del plancton marino (1950). Neunes (1965) has published a simple key for common pelagic Mediterranean copepods which may be used for the identification of the most frequent copepods even by relatively inexperienced persons.

PELECYPODS

(= bivalves = acephalons = lamellibranchs)

External Morphology

Bilaterally symmetrical molluscs. Body without a distinct head (acephalons), enclosed in a shell composed of two valves (bivalves) hinged together along one edge (dorsal hinge). On each side the tegument forms a mantle whose outer edge secretes a shell and adheres to it; the inner side of the mantle delimits the pallial or mantle cavity which is in open communication with the surrounding water. It houses two large, lamellar gills (lamellibranchs). The visceral mass and the foot are situated ventrally; the foot is hatchet-shaped (pelecypods) and used to dig into the substrate or to adhere by elastic attachment threads (byssus) to hard objects. In the burrowing forms the two edges of the mantle are fused, except at the level of the foot, and form two siphons, one of which (inhalant, or ventral siphon) allows the water to enter the pallial cavity, and the other (exhalant, or dorsal siphon) ejects the water together with the faeces.

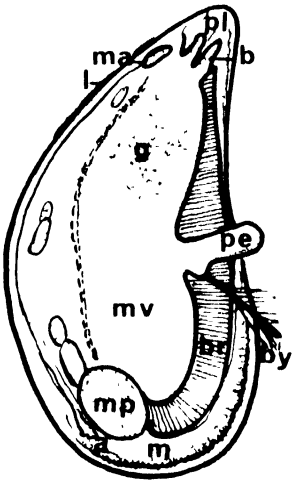


Figure 1:
Mussel after removal of valve, mantle and gills of right side

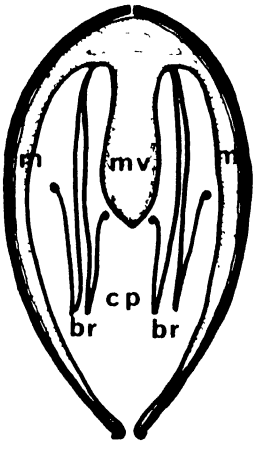


Figure 2
Transverse section of a mussel

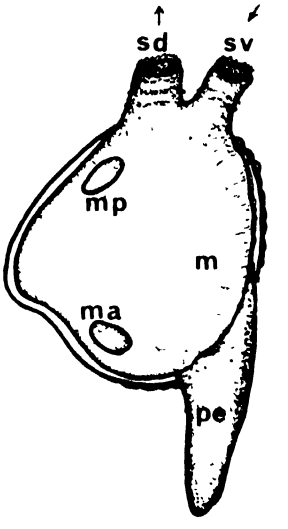


Figure 3:
Burrowing form: section after removal of left valve

Legend: a = anus, b = mouth, br = gill, by = byssus, cp = pallial cavity, g = digestive gland, l = ligament, m = mantle, ma = anterior adductor muscle, mp = posterior adductor muscle, mv = visceral mass, pl = labial palps, pe = foot, sd = dorsal siphon, sv = ventral siphon

Fig. 1. External morphology of a bivalve.

General Remarks and Technical Terms

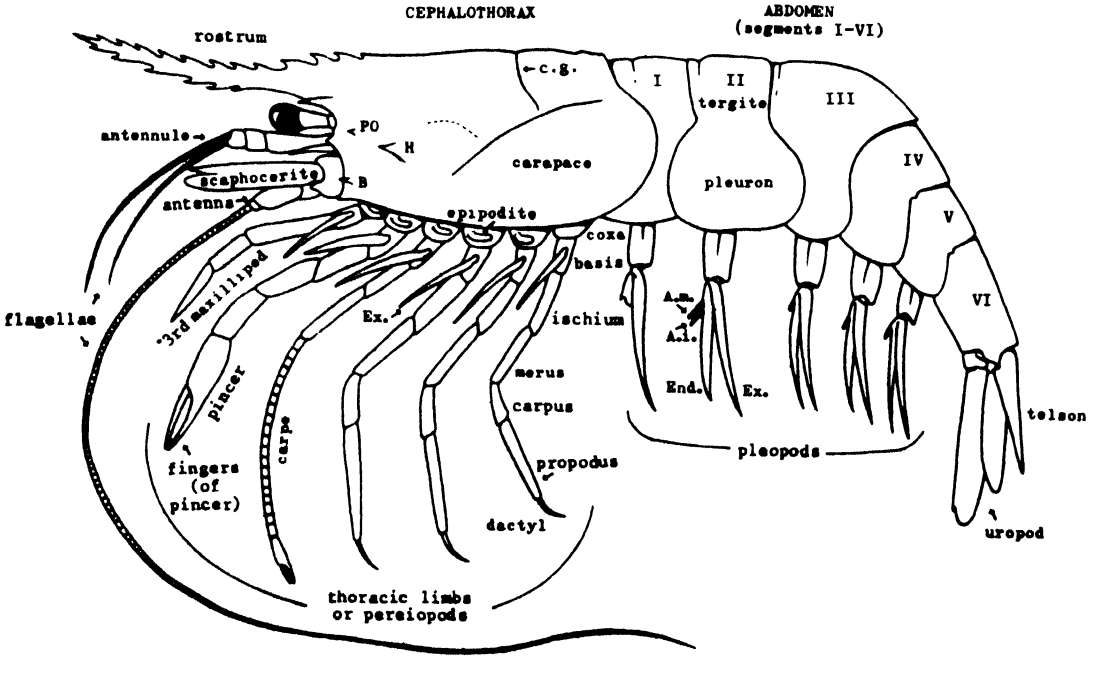
All crustaceans of commercial importance occurring in the Mediterranean and the Black Sea belong to the Order Decapoda (decapods), with the exception of the mantis shrimps which are Stomatopoda (stomatopods).

DECAPODS

The decapods can be summarily defined as crustaceans with a body divided into two regions:

(a) A cephalothorax bearing stalked eyes and 13 pairs of appendages: antennulae, antennae, mandibles, first and second maxillae, first, second and third mandibular feet (or maxillipeds), and five pairs of thoracic limbs of which generally one or several (chelipeds) terminate in pincers (or chelae), while the remaining are walking legs.

(b) An abdomen, typically composed of six segments, each of which bears one pair of appendages (pleopods) and of a terminal piece, the telson.



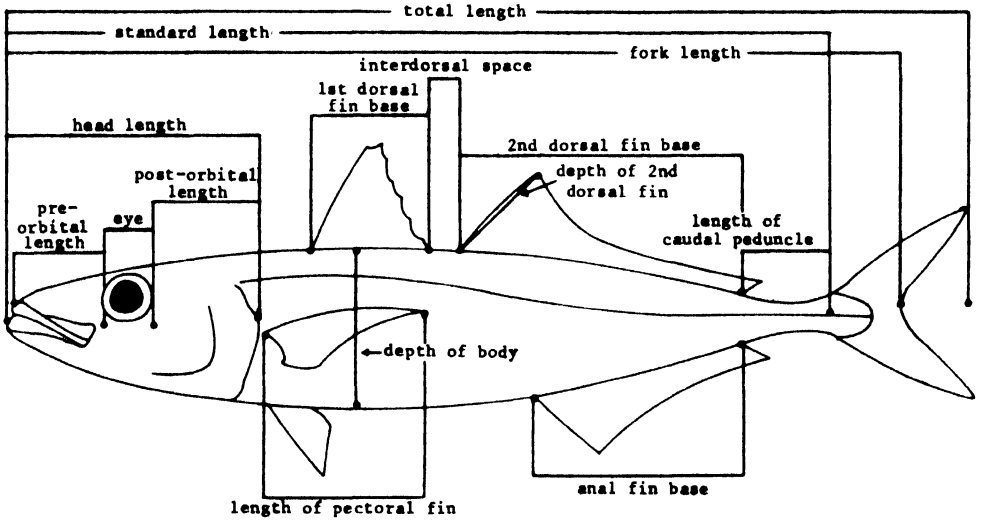
Diagrammatic design of a shrimp: the terminology used in the description is indicated on this explanatory design representing a Natantia (male carid shrimp). Although there are evident analogies between Macrura Natantia (shrimps) and Reptantia (lobsters), it should be borne in mind that the Brachyura (crabs) look rather different due to the great reduction of the abdomen which is folded under a well developed and broad cephalothorax.

Abbreviations: c.g. = cervical groove; B = branchiostegal spine; H = hepatic spine; PO = postorbital spine; EX = exopodite; End. = endopodite; A.i. = appendix interna; A.m. = appendix masculina

Fig. 2. External morphology of a decapod.

Principal Measurements Used

(shortest distance between the points marked: •)



General Nomenclature of the External Morphology

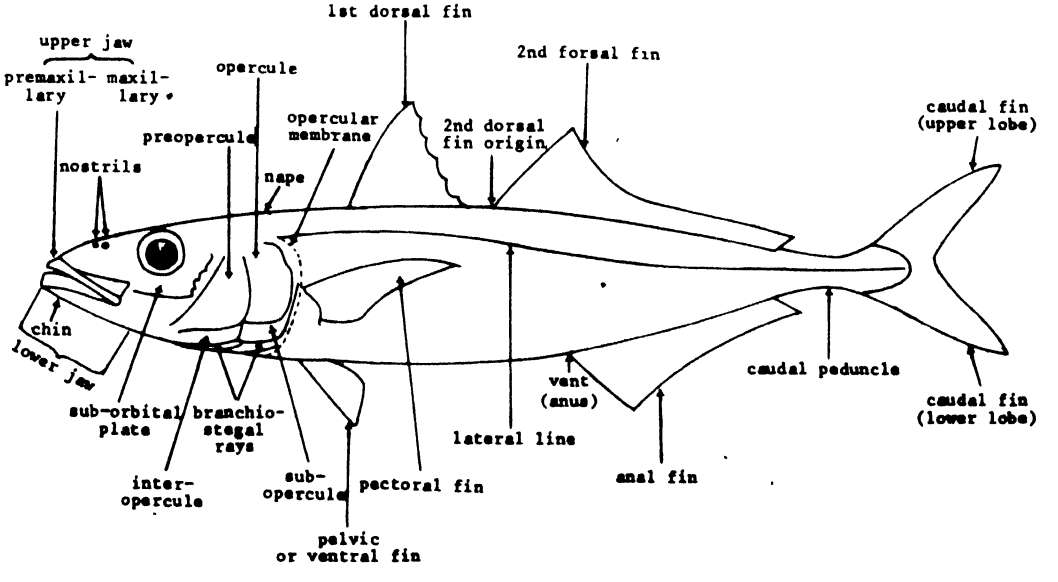


Fig. 3. Principal measurements used and general nomenclatureof the external morphology of bony fishes.

MYTIL Mytil 1
1972

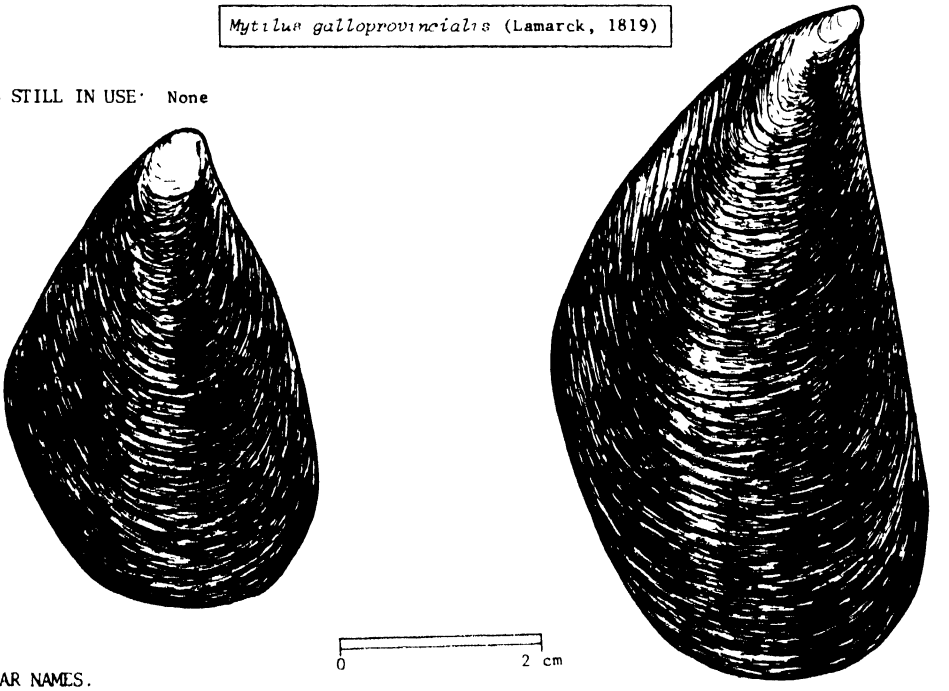
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: MYTILIDAE

Fishing Area 37
(Medit. and Black Sea)

Mytilus galloprovincialis (Lamarck, 1819)

SYNONYMS STILL IN USE: None



VERNACULAR NAMES.

FAO - En : Mediterranean mussel
Fr : Moule méditerranéenne
Sp : Mejillón mediterráneo

NATIONAL - ALBN:		ISRL:	Zidpit galit	ROMN:	Midie
ALGR.	Babbouch	ITAL:	Mitilo	SPAN:	Mejillón
BULG:	Cherna mida	LEBN:		SYRI:	
CYPR:	Mydia	LIBY:		TUNS:	Tamr el bahr
EGYP:		MALT:	Masklu	TURK:	Midye
FRAN:	Moule	MONC:	Mula	USSR:	Midia
GREC:	Mýdi	MORC:	Moule	YUGO:	Dagnja

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Shell elongate, sub-quadrangular, *equivalve, blackish-violet in colour; umbones (beaks) terminal, pointed* and slightly curved forward; ventral surface of valves flattened behind the beaks; *ventral margin of the shell straight, posterior margin rounded, ligamentary margin forming a distinct angle. Inner side of valves smooth, bearing a large scar which extends dorsally and posteriorly* (consisting of an elongated portion: traces of retractor muscles of foot, medium and posterior retractor muscles of byssus, and a circular posterior portion: trace of posterior adductor muscle). There are also two small anterior muscular scars (traces of the anterior adductor muscle and of the anterior retractor muscle of byssus). Hinge formed by 3 to 4 small teeth.

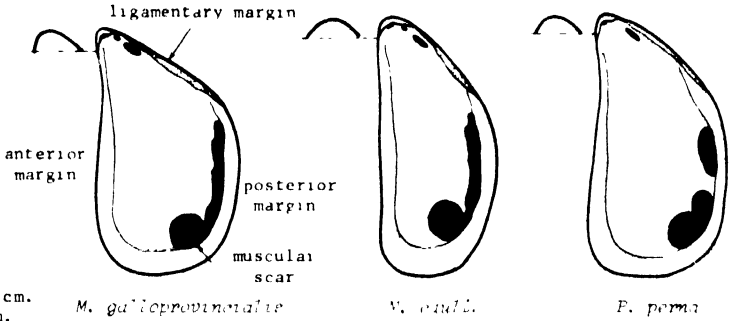
Other field characters: edge of mantle violet or purplish-violet in colour.

Fig. 4. Identification sheet of Mytilus.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA

Mytilus edulis has a less prominent ligamentary margin, the ventral surface of the valves is not flattened behind the beak. Its shape is often relatively more elongate.

Perna (Mytilus) perna, which occurs along the coasts of Algeria and northern Tunisia, has a pearly shell; the scar of the retractor muscles of the byssus is distinct from that of the posterior adductor muscle.



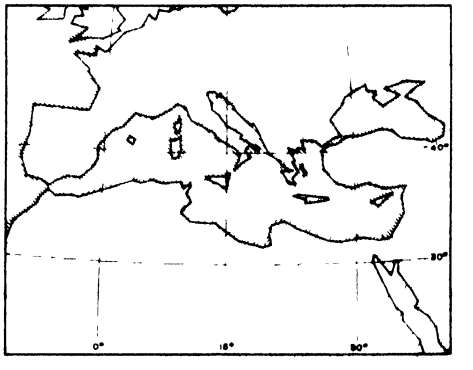
M. galloprovincialis *M. edulis* *P. perna*

SIZE

Maximum - length. 10-11 cm.
Common - length: 7-8 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR

A very common species with a wide range: western and eastern Mediterranean (though rare along the North African coast), western part of the English Channel and Atlantic coasts of France, Portugal and Spain.



Lives at the upper limit of the infralittoral zone, attached to hard substrates (rocks, poles, boulders, piers, buoys). It is cultivated on a large scale.

Feeds on phytoplankton and suspended organic particles. The sexes are distinct and spawning extends throughout the year.

PRESENT FISHING GROUNDS

Mytiliculture is practised in the Mediterranean (Italy, France, Spain, Tunisia, Turkey and Yugoslavia) and in the Atlantic. In the Mediterranean the specimens are attached by their byssus (attachment threads emerging from between the anterior margins of the shell) to strings suspended to frameworks or buoys.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION

Separate statistics for this species are collected in Bulgaria, Italy (1971: 18 200 tons), France (1971: 8 900 tons), Spain, Tunisia and Turkey, the production reported in 1971 for the Mediterranean and Black Sea totalling 28 200 tons.

On natural banks it is taken with rakes and dredges, but the main production comes from mytiliculture.

Its commercial value is high, as the flesh is very highly esteemed. It is consumed raw or cooked.

Fig. 4. (cont'd)

PORT Carc 1
1972

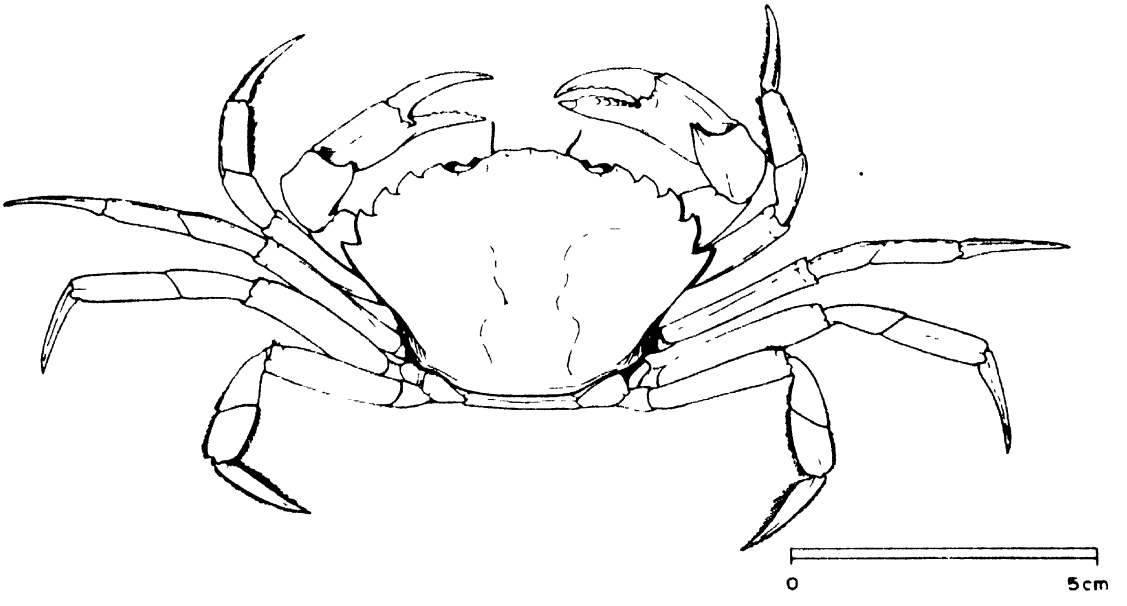
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: PORTUNIDAE

Fishing Area 37
(Medit. and Black Sea)

Carcinus mediterraneus Czerniavsky, 1884

SYNONYMS STILL IN USE: None



VERNACULAR NAMES

FAO - En : Mediterranean shore crab
Fr : Crabe vert de la Méditerranée
Sp : Cangrejo verde del Mediterráneo

NATIONAL -	ALBN:		ISRL:		ROMN:	Crab
	ALGR:		ITAL:	Granchio riparıo	SPAN:	Cangrejo de mar
	BULG:	Krivrak	LEBN:		SYRI:	
	CYPR:	Kavouros	LIBY:		TUNS:	Oum jniba
	EGYP:		MALT:	Granc tal-marsa	TURK:	Çingene pavuryasí
	FRAN:	Crabe vert	MONC:	Grita verde	USSR:	Iravjanoi kzab
	GREC:	Kavouras	MORC:	Crabe	YUGO:	Kosmej susa

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Carapace hexagonal, its upper side smooth, slightly hairy, divided into discrete regions; *frontal border cut into 3 lobes of which the median is slightly prominent; antero-lateral margins cut into 5 teeth* (including orbital angle), *the last one being the sharpest.* Chelipeds strong, slightly assymmetrical, smooth, with only one strong and sharp tooth on the anterior region of the carpus. Walking legs long and rather slender; in the last pair the propodus is more strongly flattened than in the preceding legs, and also the dactyl is flattened and lanceolate. Colour variable; the upper side is generally deep green in adult specimens, while the underside is tinged with yellow or red.

Fig. 5. Identification sheet of Carcinus.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA.

This species has been confused until very recently with *Carcinus maenas*, a well-known Atlantic species. The two species, which are very similar, can be distinguished by a number of relatively minor characters: *C. maenas* has a carapace of granular surface, less sharp antero-lateral teeth and strongly reduced hair cover. The adult males of both species show, however, a striking differential feature; in *C. mediterraneus*, the first pair of sexual pleopods, well visible when the abdomen is lifted up, are straight and parallel, while they are strongly curved and divergent in *C. maenas*.

pleopods

C. mediterraneus male

C. maenas male

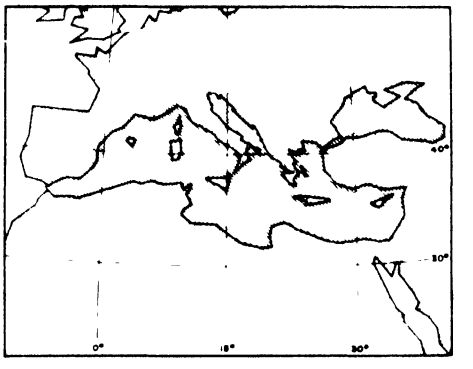
SIZE

Maximum: 5 cm length; about 6 cm width.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR

Present throughout the Mediterranean and the Black Sea. In the Atlantic it is known to occur around the Canary Islands. It should be noted that *C. maenas* is present mainly in the eastern Atlantic, from Norway to Mauritania.

Inhabits shallow waters and brackish lagoons.



PRESENT FISHING GROUNDS.

Coastal areas of the continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION.

Separate statistics for this species are collected only in Spain (1971: 100 tons). The catch of "miscellaneous marine crabs" reported in 1971 for the Mediterranean and the Black Sea totalled 800 tons.

Caught with beach seines, bottom trawls and pots.

Marketed fresh.

Fig. 5. (cont'd).

1972

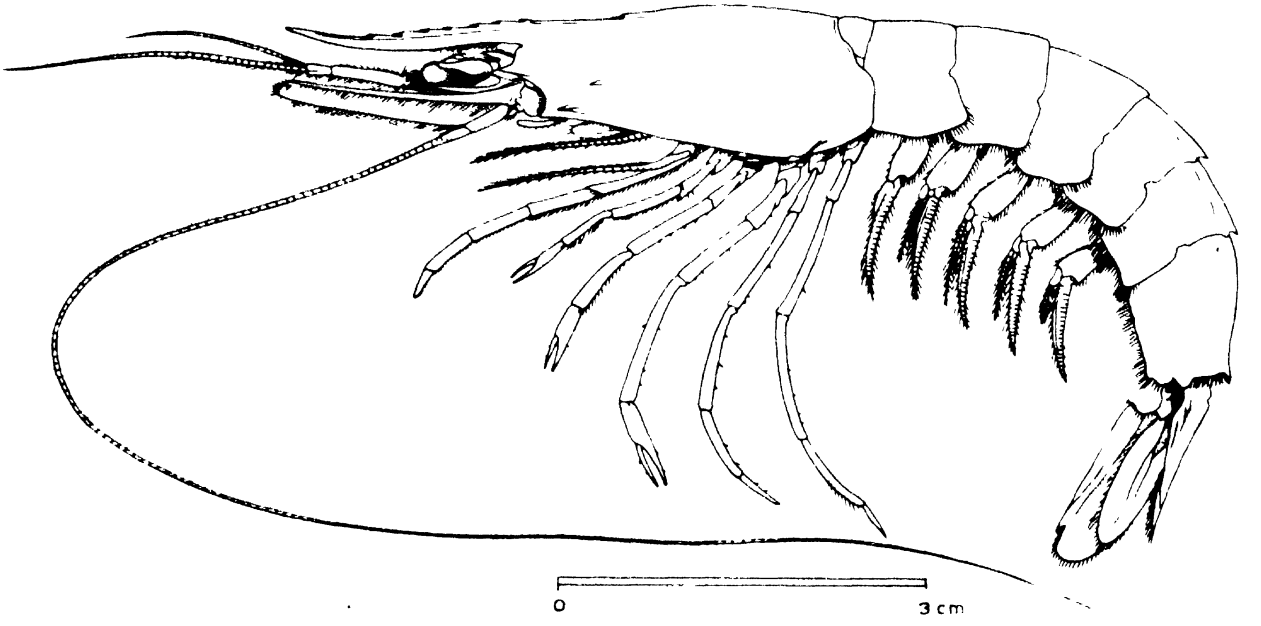
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: PENAEIDAE

Fishing Area 37
(Medit. and Black Sea)

Parapenaeus longirostris (Lucas, 1846)

SYNONYMS STILL IN USE: None



VERNACULAR NAMES

FAO - En : Deep-water pink shrimp
Fr Crevette rose du large
Sp . Gamba de altura

NATIONAL -	ALBN:		ISRL:	Bar-penon	ROMN:	
	ALGR:		ITAL:	Gambero rosa	SPAN.	Gamba
	BULG:		LEBN:	Kraïdes	SYRI:	
	CYPR:	Garida	LIBY:	Gambari ahmar	TUNS:	Gambri abiedh
	EGYP:	Gamberi agouz	MALT:	Gamblu mezzan	TURK:	Karides
	FRAN:	Crevette rose	MONC:		USSR:	Krevetka
	GREC:	Garidáki	MORC:	Crevette rose	YUGO:	Kozica golema

DISTINCTIVE CHARACTERS AND DIAGNOSIS

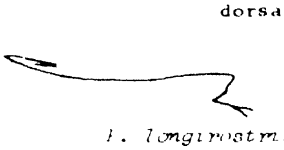
Carapace covered with very short and hardly visible hairs; a sutural line extends over the whole length of the sides behind the eye. *Rostrum sinuous*, its basal portion pointing slightly downward, but its tip bent upward; *its lower border is unarmed, while the upper border bears 7 to 8 teeth; another dorsal tooth is inserted far backward on the post-rostral keel* which extends almost to the hind border of the carapace; hepatic spine present.

Fig. 6. Identification sheet of Parapenaeus.

Other field characters: abdominal segments I to III without keels; segments IV to VI with a dorsal keel increasing gradually in size, each armed with a small and sharp posterior tooth. Telson with a deep longitudinal depression on its upper side. Both antennular flagellae are long.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA

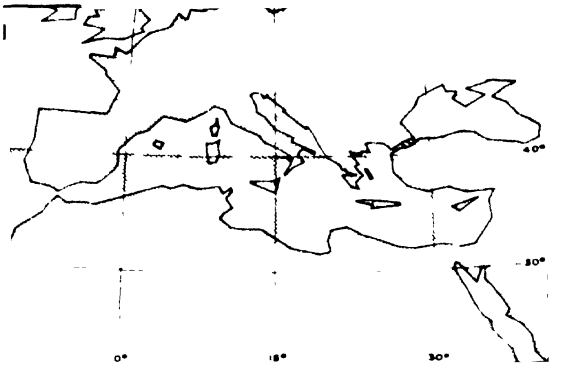
The shape of the rostrum and the particular arrangement of rostral teeth distinguish this species at first sight from the other Mediterranean penaeids; the presence of an isolated dorsal tooth, far behind the true rostral teeth, is also very characteristic.



P. longirostris
rostrum and anterior part of carapace

SIZE:

Maximum: 12 cm, common: 8 to 10 cm.



GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR

Present throughout the Mediterranean and the Sea of Marmara, but absent from the Black Sea. Also found in the Atlantic Ocean, on the eastern side from Portugal to Angola, and on the western side from Massachussets to Venezuela.

Inhabits mostly muddy or muddy-sand bottoms between 100 and 400 m depth, but may be found from 50 to 700 m.

PRESENT FISHING GROUNDS

Continental shelf and slope.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION

No separate statistics for this species are collected within the area. Data for the statistical category "shrimps" are collected however in Algeria, Egypt, France, Greece, Italy (1971: 9 800 tons), Morocco, Spain (1971: 2 200 tons), Tunisia and Turkey. The catch reported in 1971 by these countries totalled 17 500 tons.

Caught with bottom trawls.

Marketed fresh.

Fig. 6. (cont'd).

1972

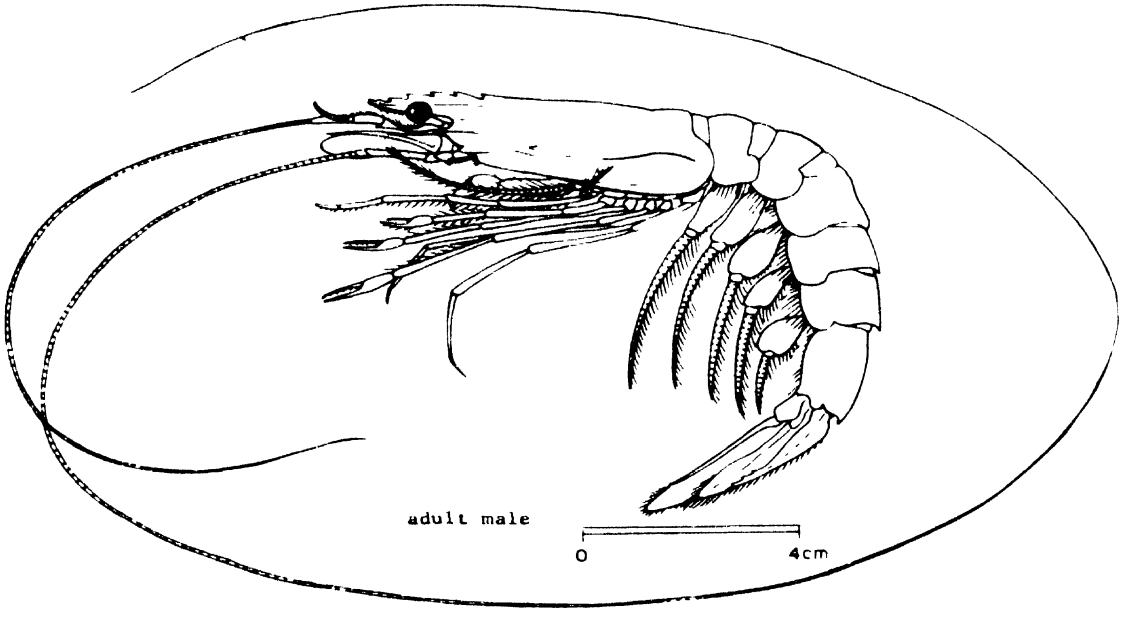
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: PENAEIDAE

Fishing Area 37
(Medit. and Black Sea)

Aristeomorpha foliacea (Risso, 1827)

SYNONYMS STILL IN USE: None



VERNACULAR NAMES:

FAO - En : Giant red shrimp
Fr : Crevette rouge
Sp : Langostino moruno

NATIONAL -	ALBN:		ISRL:	Ariston adom	ROMN:		
	ALGR:	Kam'rûn	ITAL:	Gambero rosso	SPAN:	Langostino moruno	
	BULG:		LEBN:		SYRI:		
	CYPR:	Garida	LIBY:		TUNS:	Gambri ahmar	
	EGYP:		MALT:	Gamblu tal-fond	TURK:	Karides	
	FRAN:	Crevette rouge	MONC:	Gambaru de fundu	USSR:		
	GREC:	Garída	MORC:	Crevette royale	YUGO:	Kozika crvena	

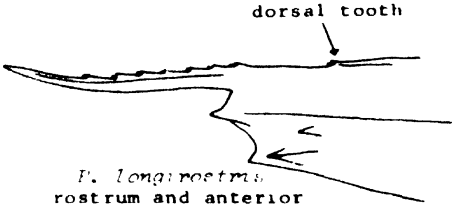
DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Carapace covered with short hair; a prominent keel runs along the lower border on each side of the carapace and another keel, slightly oblique, along its posterior half. *In females and young males the rostrum is long and strongly bent upward; its upper margin bears 5 or 6 rather strong teeth along its base, and some smaller denticles which extend near to the tip.* In adult males the rostrum is much shorter and bears only the 5 or 6 basal teeth. *A strong hepatic spine is present.* Colour: wine-red with a violet tinge on the upper side of the carapace.

Aristeomorpha foliacea
rostrum of female and young male

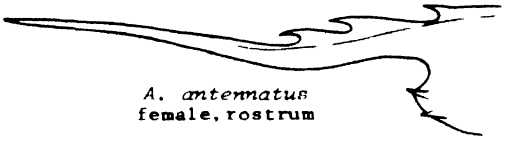
Fig. 7. Identification sheet of Aristeomorpha.

Other field characters. abdominal segments I and II not keeled; the following ones bear a dorsal keel which increases in size and is prolonged backward in a small tooth. Telson with a median longitudinal groove. Upper flagellum of antennulae short and flattened.



P. longirostris
rostrum and anterior part of carapace

A. antennatus
male, rostrum



A. antennatus
female, rostrum

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA.

Parapenaeus longirostris is the only Mediterranean penaeid having a certain resemblance to *A. foliacea*. However, this similarity only applies to females and young males, as the adult males of *A. foliacea* have a rostrum which is much shorter than that of *P. longirostris*. In any case, the absence of a dorsal tooth in *A. foliacea* which is present in *P. longirostris* allows an easy distinction of this species from the other; in addition, *P. longirostris* is pink-coloured.

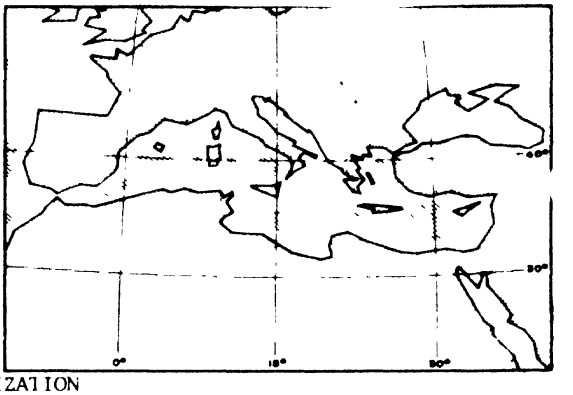
A related species, *Aristeus antennatus*, shows the same sexual dimorphism with regard to the extension of the rostrum, but bears only 3 rostral teeth (5 to 6 in *A. foliacea*) and is of a darker wine-red colour.

SIZE

Maximum: 22 cm; **common:** 15 to 18 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR·

Present throughout the Mediterranean but absent from the Black Sea. Also found in the eastern Atlantic from the Bay of Biscay to Rio d'Oro, in South Africa, Japan, and Australia.



Inhabits muddy bottoms, mainly between 250 and 350 m, but has been caught up to depths of 1 300 m.

PRESENT FISHING GROUNDS·

Deep areas of the continental shelf and the slope.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION

No separate statistics for this species are collected within the region. Data for the statistical category "shrimps" are collected however in Algeria, Egypt, France, Greece, Italy (1971: 9 800 tons), Morocco, Spain (1971: 2 200 tons), Tunisia and Turkey. The catch reported in 1971 by these countries totalled 17 500 tons.

Caught with bottom trawls.

Marketed fresh.

Fig. 7. (cont'd).

PEN Pen 1

1972

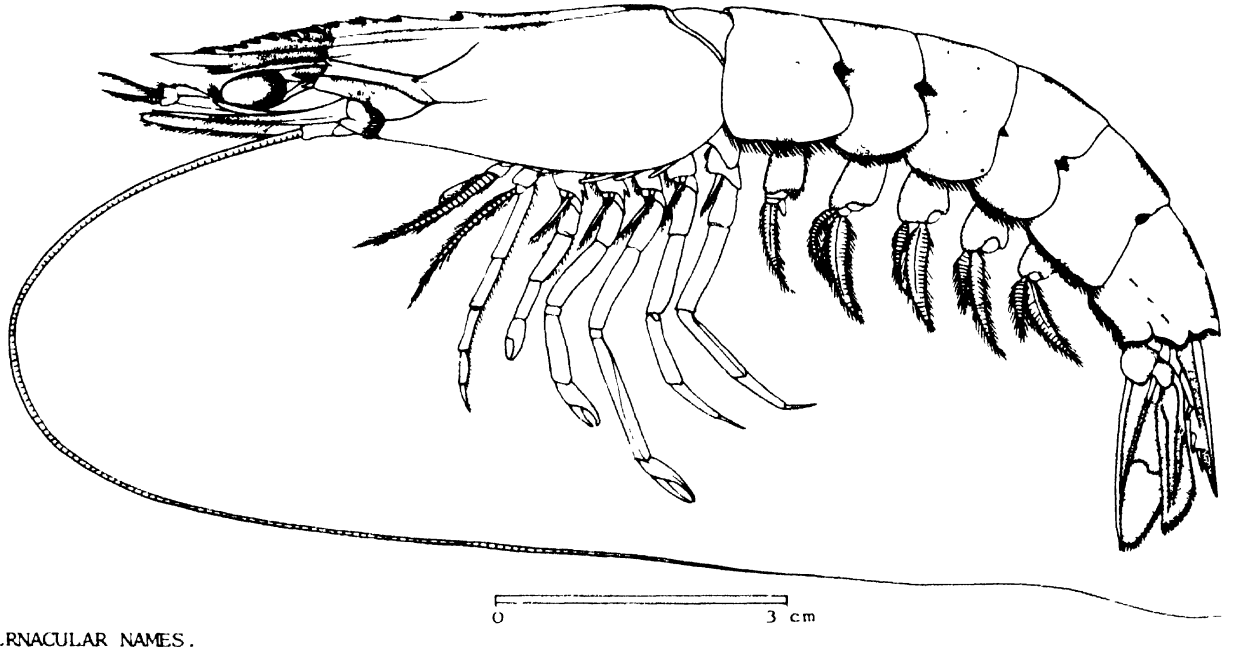
FAO SPECIES IDENTIFICATION SHEETS

FAMILY. PENAEIDAE

Fishing Area 37
(Medit. and Black Sea)

Penaeus kerathurus (Forskål, 1775)

SYNONYMS STILL IN USE: *Penaeus trisulcatus* Leach, 1815



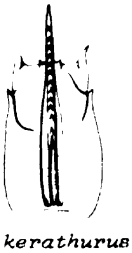
VERNACULAR NAMES.

FAO - En . Triple-grooved shrimp
Fr : Caramote
Sp : Langostino

NATIONAL -	ALBN:		ISRL:	Penon telat-harizi	ROMN:		
	ALGR:	Caramote	ITAL:	Mazzancolla	SPAN:	Langostino	
	BULG:		LEBN:		SYRI:	Kreidès	
	CYPR:		LIBY:	Gambari	TUNS:	Gambri kbeir	
	EGYP:	Gambari azzari	MALT:	Gamblu mperjali	TURK:	Karides	
	FRAN:	Caramote	MONC:	Gambaru grossu	USSR:	Krevetka	
	GREC:	Garída	MORC:	Bouquet	YUGO:		

DISTINCTIVE CHARACTERS AND DIAGNOSIS.

Carapace strongly calcified, with many grooves on its upper side and on the anterior halves of the sides. *Rostrum strong, extending a little beyond the eyes, armed with one ventral tooth and 10 dorsal teeth which run backward to the middle of the carapace,* behind the last tooth, the median keel divides into 2 branches, leaving a narrow groove between them. *On each side of the midline there is a high and sharp crest which extends to the rostrum,* thus two deep dorsal grooves can be seen running along the carapace throughout its length. A strong hepatic spine is present. The colouration is rather characteristic: there are transverse dark bands or blotches on a lighter background.



Penaeus kerathurus
dorsal view of
carapace and rostrum

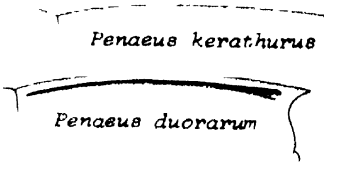
Fig. 8. Identification sheet of Panaeus kerathurus.

Other field characters: abdominal segments I to III without a keel; each of the segments IV to VI bear a keel which increases in sharpness; that of segment VI ends in a small tooth. Telson with a dorsal groove formed by a pair of longitudinal, sharp keels. Both antennular flagellae are very short.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

The other Mediterranean penaeids can be distinguished from *P. kerathurus* by their different colouration, and by the absence of a tooth on the lower border of the rostrum, of a rostral keel bifid posteriorly and of a pair of secondary keels parallel to the midline.

P. duorarum, a species which is common in the tropical Atlantic off Africa, differs from *P. kerathurus* in the presence of a deep groove on each side of the dorsal keel of the abdominal segment VI.



6th abdominal segment (lateral view)

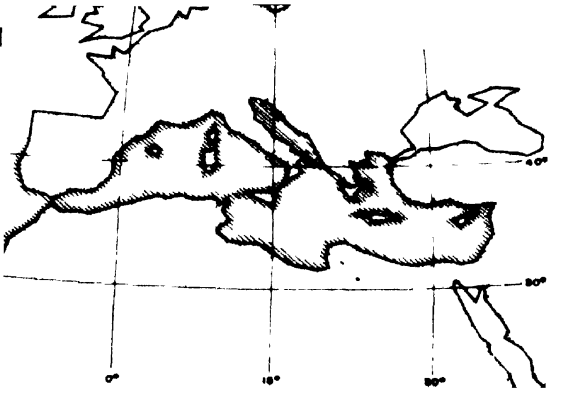
SIZE:

Maximum: about 20 cm; common: 14 to 16 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR.

Present throughout the Mediterranean, but absent from the Black Sea. Also found in the eastern Atlantic from Portugal to Angola.

Inhabits relatively shallow waters, down to about 50 to 70 m, over sandy-mud bottoms.



PRESENT FISHING GROUNDS:

Continental shelf and often in the vicinity of estuaries into which penetrate the juveniles.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION.

Separate statistics for this species are collected only in Italy (1971: 3 900 tons) and Spain (1971: 100 tons). This species is included in other countries in the statistical category "shrimps for which the reported catch in 1971 in the Mediterranean and the Black Sea totalled 17 500 tons.

Caught with trawls and occasionally with pots.

Marketed fresh.

Fig. 8 (cont'd).

MUL Mull 1

1971

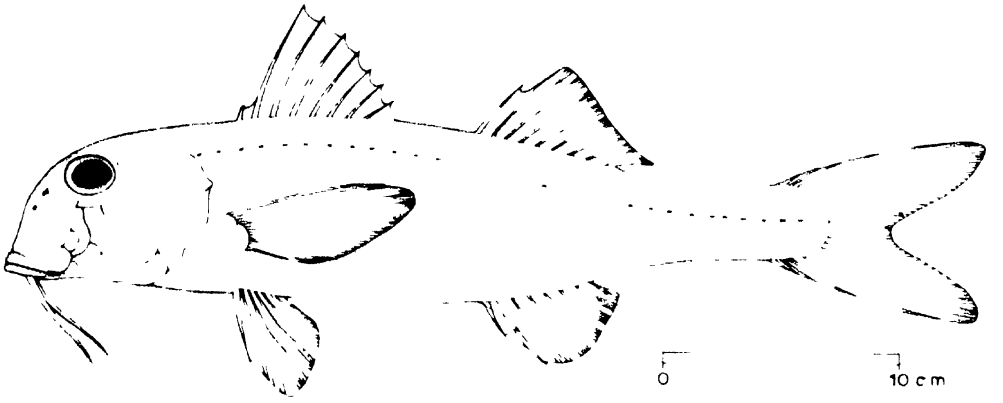
FAO SPECIES IDENTIFICATION SHEETS

FAMILY MULLIDAE

Fishing Area 37
(Medit and Black Sea)

Mullus barbatus Linnaeus, 1758

SYNONYMS STILL IN USE None



VERNACULAR NAMES

FAO - En - Striped mullet
Fr - Rouget barbet
Sp Salmonete de fango

NATIONAL -	ALBN:		ISRL:	Mulit adumma	ROMN:	Barbun
	ALGR:	Mlāl el khez	ITAL:	Triglia di fango	SPAN:	Salmonete de fango
	BULG:	Barbunja	LEBN:	Sultan ibrahim ramli	SYRI:	Sultan ibrahim ramli
	CYPR:	Strillia	LIBY:		TUNS:	Mellou
	EGYP:	Barbouni	MALT:	Trilja tal-hama	TURK.	Barbunya
	FRAN:	Rouget barbet	MONC:	Treglia d'arga	USSR·	Sultanka
	GREC:	Koutsomoúra	MORC.	Rouget	YUGO:	Trlja od blata

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Deep and short head (head length 4.8 to 5 times in total length) with a *pair of long barbels* under the chin; *profile of the snout nearly vertical, the mouth reaching to the level of the eyes; under the orbit, two large scales, preceded by a smaller one* (suborbital scales); *upper jaw toothless;* back and sides reddish-pink *without yellow longitudinal bands, first dorsal fin without dark spots or bands.*

Other field characters: body rather compressed from side to side; two well separated dorsal fins, the first with 8, sometimes 9 spines, the second with 1 spine and 8 soft rays; scales large and easily detached.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA.

Mullus surmuletus: differs from *M. barbatus* by the oblique (not nearly vertical) profile of the snout, the slightly longer head (head length 4 to 4.3 times in total length), the mouth not reaching to the level of the eye, the presence of 2 suborbital scales only, of yellow bands along the lower sides

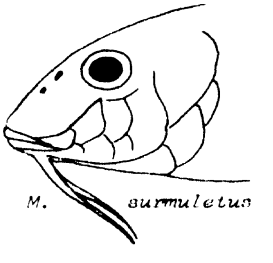


Fig. 9. Identification sheet of Mullus barbatus

Upeneus moluccensis and *U. asymmetricus* differ from *M. barbatus* by the presence of teeth on the upper jaw and of oblique dark bars on the caudal fin.

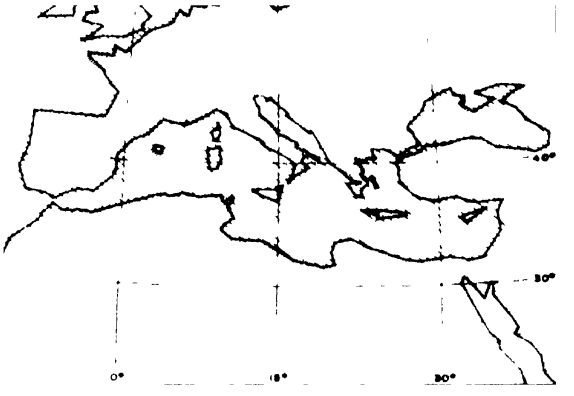
U. moluccensis

SIZE:

Maximum: 30 cm; common. 10 to 15 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Common in coastal waters throughout the Mediterranean, the Black Sea and the Azov Sea. The population in the Black and Azov Seas is considered a subspecies, *(M. barbatus ponticus)*; in the eastern Atlantic the species occurs from the British Isles to the coast of Senegal.



Inhabits usually shallow sand and mud bottoms, but may occur down to depths of 300 m.

Feeds predominantly on small bottom-living invertebrates (crabs, worms, etc.)

PRESENT FISHING GROUNDS.

Continental shelf, usually on trawling grounds at depths between 20 and 200 m

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

M. barbatus and *M. surmuletus* are included in a single statistical category in Cyprus, Egypt (1967: 1 500 tons), France, Greece, Italy (1970. 7 600 tons), Israel, Malta, Spain (1970: 2 200 tons), Tunisia (1967: 9 700 tons), Turkey (1969 3 500 tons), USSR and Yugoslavia, the catches in the area estimated for 1970 on the basis of these data totalling 28 600 tons.

Caught mainly with bottom trawls, occasionally with beach seines and gill nets.

Highly esteemed food fish, marketed fresh or frozen.

Fig. 9. (cont'd).

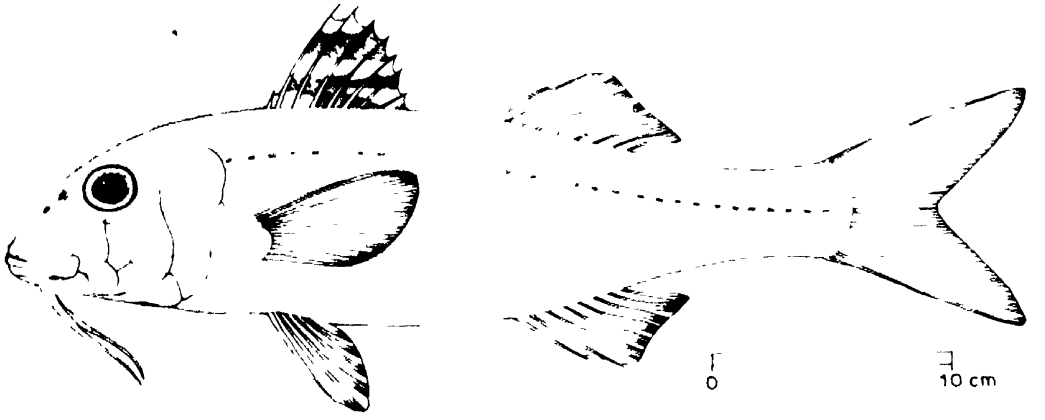
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: MULLIDAE

Fishing Area 37
(Medit. and Black Sea)

Mullus surmuletus Linnaeus, 1758

SYNONYMS STILL IN USE. None



VERNACULAR NAMES

FAO - En : Red mullet
Fr : Rouget de roche
Sp : Salmonete de roca

NATIONAL -	ALBN:		ISRL.	Mulit happassim	ROMN.	
	ALGR:	Mlal el hjar	ITAL:	Triglia di scoglio	SPAN:	Salmonete de roca
	BULG:	Pastra barbunja	LEBN:	Sultan ibrahim sakhri	SYRI:	Sultan ibrahim sakhri
	CYPR:	Barbouni	LIBY:		TUNS.	Mellou
	EGYP:	Barbouni	MALT:	Trilja tal-qawwi	TURK.	Tekir
	FRAN:	Rouget de roche	MONC:	Treglia de scoegliu	USSR:	Barbunya
	GREC:	Barbouni	MORC.	Rouget	YUGO:	Trlja od kamena

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Deep and short head (head length 4 to 4.3 times in total length) with *a pair of long barbels under the chin; the mouth does not reach the level of the anterior margin of the eyes, upper jaw toothless;* under the orbit, two large scales (suborbital scales); back and sides reddish to scarlet red; *lengthwise yellow bands on each side.*

Other field characters: body rather compressed from side to side; two well separated dorsal fins, the first normally with 8 spines, the second with one spine and 8 soft rays; scales large and easily detached.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

Mullus barbatus differs from *M. surmuletus* by the nearly vertical profile of the snout, the slightly longer head (head length 4.8 to 5 times in total length), the mouth reaching to the level of the eyes, the presence of 3 suborbital scales (two large scales preceded by a smaller one) and the absence of yellow bands along the sides of the body and of dark spots or bands on the first dorsal fin.

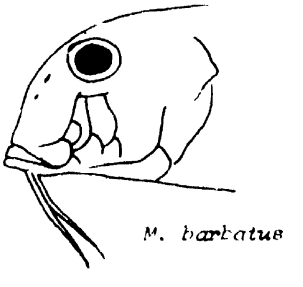
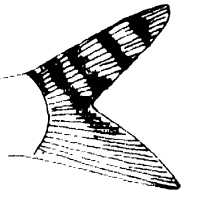


Fig. 10. Identification sheet of Mullus surmuletus.

Upeneus moluccensis and *U. asymmetricus* differ from *M. surmuletus* by the presence of teeth on the upper jaw and of oblique dark bands on the caudal fin.



U. moluccensis

SIZE:

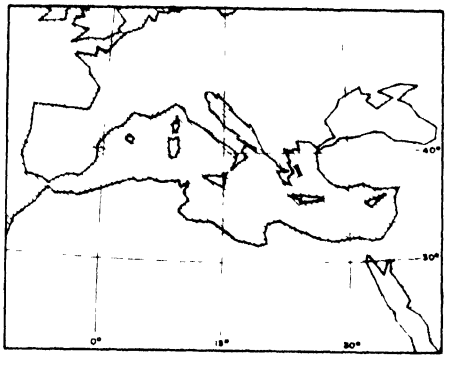
Maximum: 40 cm; common: 10 to 25 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Common in coastal waters throughout the Mediterranean and in the eastern Atlantic from the British Isles to the coast of Senegal; absent from the Black Sea.

Inhabits shallow sand, gravel and rock bottoms, generally not deeper than 90 m.

Feeds on tiny bottom-living invertebrates and small fishes.



PRESENT FISHING GROUNDS:

Shallow coastal waters of the continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

M. surmuletus and *M. barbatus* are included in a single statistical category in Cyprus, Egypt (1967: 1 500 tons), France, Greece, Italy (1970: 7 600 tons), Israel, Malta, Spain (1970: 2 200 tons), Tunisia (1967: 9 700 tons), Turkey (1969: 3 500 tons), USSR and Yugoslavia, the catches in the area estimated for 1970 on the basis of these data totalling 28 600 tons.

Caught with bottom trawls, gill nets, beach seines and traps.

Highly esteemed food fish, marketed fresh or frozen.

Fig. 10. (cont'd).

MULL Mull 2
1971

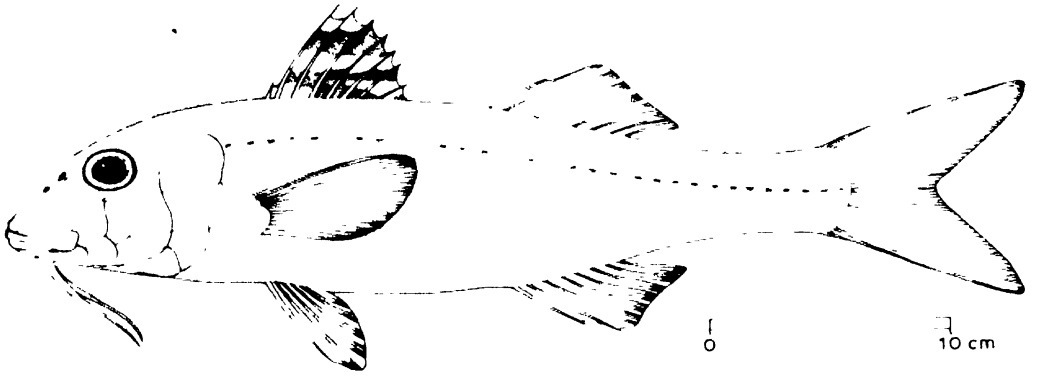
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: MULLIDAE

Fishing Area 37
(Medit. and Black Sea)

Mullus surmuletus Linnaeus, 1758

SYNONYMS STILL IN USE: None



VERNACULAR NAMES

FAO - En : Red mullet
Fr . Rouget de roche
Sp · Salmonete de roca

NATIONAL -	ALBN:		ISRL:	Mulit happassim	ROMN:	
	ALGR:	Mlal el hjar	ITAL:	Triglia di scoglio	SPAN:	Salmonete de roca
	BULG:	Pastra barbunja	LEBN·	Sultan ibrahim sakhri	SYRI:	Sultan ibrahim sakhri
	CYPR:	Barbouni	LIBY.		TUNS:	Mellou
	EGYP:	Barbouni	MALT:	Trilja tal-qawwi	TURK.	Tekir
	FRAN:	Rouget de roche	MONC.	Treglia de scoegliu	USSR:	Barbunya
	GREC·	Barbouni	MORC:	Rouget	YUGO.	Trlja od kamena

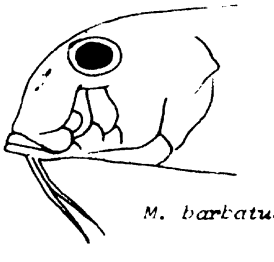
DISTINCTIVE CHARACTERS AND DIAGNOSIS·

Deep and short head (head length 4 to 4.3 times in total length) with *a pair of long barbels under the chin, the mouth does not reach the level of the anterior margin of the eyes; upper jaw toothless;* under the orbit, two large scales (suborbital scales); back and sides reddish to scarlet red; *lengthwise yellow bands on each side.*

Other field characters: body rather compressed from side to side; two well separated dorsal fins, the first normally with 8 spines, the second with one spine and 8 soft rays; scales large and easily detached.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

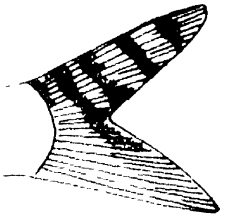
Mullus barbatus differs from *M. surmuletus* by the nearly vertical profile of the snout, the slightly longer head (head length 4.8 to 5 times in total length), the mouth reaching to the level of the eyes, the presence of 3 suborbital scales (two large scales preceded by a smaller one) and the absence of yellow bands along the sides of the body and of dark spots or bands on the first dorsal fin.



M. barbatus

Fig. 10. Identification sheet of Mullus surmuletus.

Upeneus moluccensis and *U. doggmi trieue*. differ from *M. surmuletus* by the presence of teeth on the upper jaw and of oblique dark bands on the caudal fin.



M. moluccensis

SIZE:

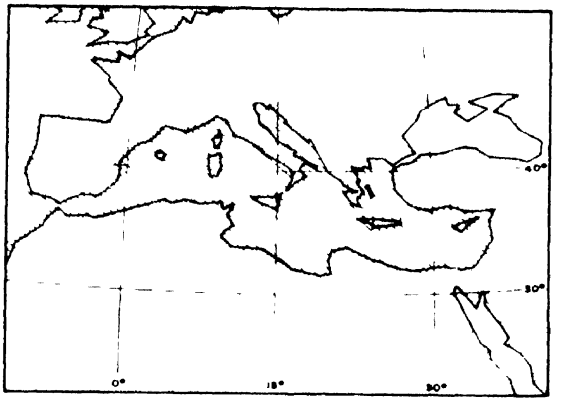
Maximum: 40 cm; common: 10 to 25 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Common in coastal waters throughout the Mediterranean and in the eastern Atlantic from the British Isles to the coast of Senegal; absent from the Black Sea.

Inhabits shallow sand, gravel and rock bottoms, generally not deeper than 90 m

Feeds on tiny bottom-living invertebrates and small fishes.



PRESENT FISHING GROUNDS.

Shallow coastal waters of the continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION.

M. surmuletus and *M. barbatus* are included in a single statistical category in Cyprus, Egypt (1967: 1 500 tons), France, Greece, Italy (1970: 7 600 tons), Israel, Malta, Spain (1970: 2 200 tons), Tunisia (1967: 9 700 tons), Turkey (1969: 3 500 tons), USSR and Yugoslavia, the catches in the area estimated for 1970 on the basis of these data totalling 28 600 tons.

Caught with bottom trawls, gill nets, beach seines and traps.

Highly esteemed food fish; marketed fresh or frozen.

Fig. 10. (cont'd).

1971

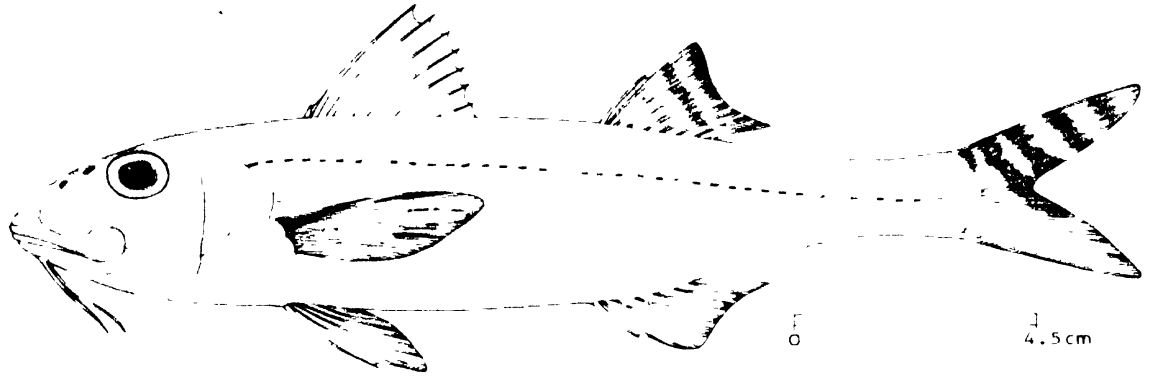
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: **MULLIDAE**

Fishing Area 37
(Medit. and Black Sea)

Upeneus moluccensis (Bleeker, 1855)

SYNONYMS STILL IN USE. None



VERNACULAR NAMES

FAO - En . Goldband goatfish
Fr . Rouget de roche
Sp : Salmonete de banda dorada

NATIONAL -	ALBN:		ISRL:	Uppon zehuv pas	ROMN:	
	ALGR:		ITAL:	Triglia dorata	SPAN:	
	BULG:		LEBN:	Sultan ibrahim yahoudi	SYRI:	
	CYPR:	Turko barbouno	LIBY:		TUNS:	
	EGYP:		MALT:	Trilja tal-lvant	TURK:	Nil barbunyasi
	FRAN:	Rouget de roche	MONC:		USSR:	
	GREC:	Lochias	MORC:		YUGO:	

DISTINCTIVE CHARACTERS AND DIAGNOSIS

Head with a pair of long barbels under the chin; upper profile of snout rather shallow and rounded; *both jaws provided with small conical teeth,* body silvery, with a *prominent yellow band from the eye to the base of the caudal fin, caudal fin with dark oblique bars.*

Other field characters: body elongate, moderately compressed from side to side; two separate dorsal fins, the first with one minute spine followed by 7 long ones, the second with 1 spine and 8 rays.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

Mullus barbatus and *M. surmuletus:* differ from *U. moluccensis* by the absence of teeth on the upper jaw, and of oblique bars on the caudal fin; also by the steeper profile of the snout.

Upeneus asymmetricus: differs from *U. moluccensis* by the absence of the yellow band along the sides of the body and of the minute first spine in the first dorsal fin.

Fig. 11. Identification sheet of Upeneus moluccensis.

SIZE:

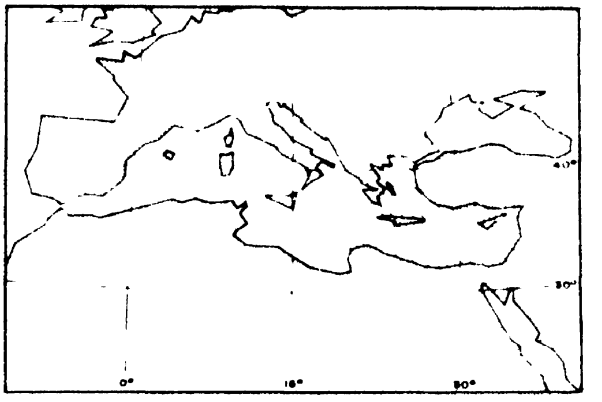
Maximum: 24 cm, common: 10 to 15 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR

Common in shallow waters in the eastern Mediterranean; absent from the western basin and the Black Sea. It has immigrated from the Red Sea through the Suez Canal, its main area of distribution being the Indian Ocean and the western Pacific.

Inhabits sandy and muddy bottoms at depths not exceeding 100 m.

Feeds on small bottom-living invertebrates.



PRESENT FISHING GROUNDS.

Shallow coastal waters of the continental shelf, usually on trawling grounds between 10 a 80 m depth

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are not reported within the area. This species may well make up 10 to 40 per cent of the total catch of mullid species in the eastern Mediterranean.

Caught mainly with trawls, also with gill nets and beach seines.

Marketed fresh or frozen.

Fig. 11. (cont'd).

SCOMBR Thun 2

1971

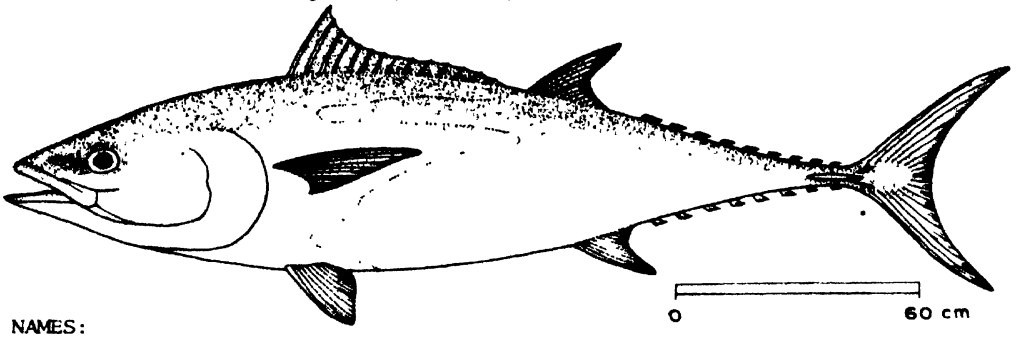
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: SCOMBRIDAE (THUNNIDAE)

Fishing Area 37
(Medit. and Black Sea)

Thunnus thynnus thynnus (Linnaeus, 1758)

SYNONYMS STILL IN USE: *Thunnus thynnus* (Linnaeus, 1758)



VERNACULAR NAMES:

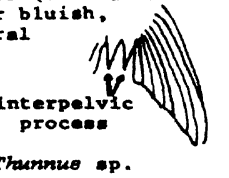
FAO - En : Bluefin tuna, Northern Atlantic
Fr : Thon rouge, Atlantique nord
Sp : Atún, Atlántico Norte

NATIONAL -	ALBN:		ISRL:	Tunna kehula	ROMN:	Ton
	ALGR:	Tunn	ITAL:	Tonno	SPAN:	Atún
	BULG:	Tunets	LEBN:		SYRI:	
	CYPR:	Tonos	LIBY:	Tonn	TUNS:	Toumahmar
	EGYP:		MALT:	Tonn	TURK:	Orkinos,
	FRAN:	Thon rouge	MONC:	Tunu	USSR:	Tunets
	GREC:	Tònnos	MORC:	Thon	YUGO:	Tun

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Large fish of fusiform and rounded body (nearly circular in cross-section), *very robust in front; finlets present behind the second dorsal and the anal fins; two dorsal fins separated only by a narrow interspace, the second higher than the first; pectoral fins very short,* never reaching the interspace between the dorsal fins; *a well developed, although not particularly conspicuous corselet* (area behind the head and around the pectoral fins covered with larger and thicker scales); *very small scales on the rest of the body;* on each side of the caudal peduncle, a strong lateral keel between two small keels located at the bases of the caudal fin lobes; back dark-blue or black, lower sides and belly silvery-white with colourless transverse lines alternated with rows of colourless dots (the latter dominate in older fish), visible only in fresh specimens; first dorsal fin yellow or bluish, *the second reddish-brown;* anal fin and finlets dusky yellow edged with black; lateral keel black in adults.

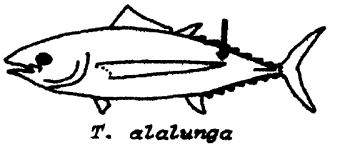
Other field characters: 9 to 10 dorsal and 8 to 9 anal finlets; eye small; 2 separate flaps (interpelvic process) between the pelvic fins; immature specimens are more slender than adults.



Thunnus sp.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

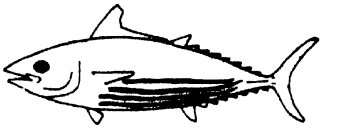
T. alalunga differs from *T. t. thynnus* by the great length of its pectoral fins, which reach backwards well beyond the end of the second dorsal fin; also by its smaller size, the lower number of finlets and its colour pattern, particularly the white-edged caudal fin.



T. alalunga

Fig. 12. Identification sheet of Thunnus thynnus.

Euthynnus alletteratus differs from *T. t. thynnus* by the relatively lesser height of the second dorsal fin (clearly lower than the first dorsal fin), by the smaller number of finlets (8 dorsal and 7 anal), by the absence of scales behind the corselet and by the peculiar striped colour pattern of the back.



K. pelamis

Katsuwonus pelamis differs from *T. t. thynnus* at first sight by the presence of very characteristic longitudinal dark bands along the lower sides.

Orcynopsis unicolor differs from *T. t. thynnus* by its laterally compressed body and the shape (straight or convex) and colour (black and white) of its first dorsal fin.

Sarda sarda differs from *T. t. thynnus* by its striped back and the greater length of its upper jaw, which reaches at least to the hind edge of the eye.

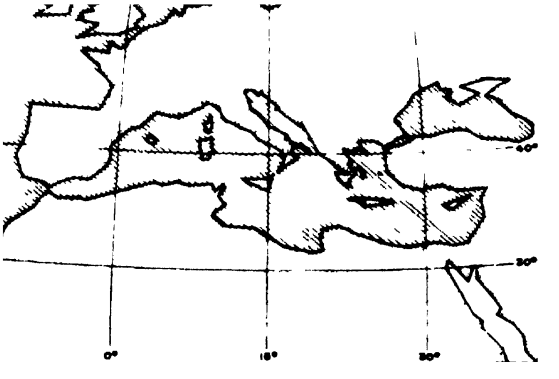
Auxis and *Scomber* species differ from *T. t. thynnus* by the large interspace between the dorsal fins (at least equal to the length of the first dorsal fin base).

SIZE:

Maximum: over 300 cm; common: 40 to 200 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Present throughout the Mediterranean and the Black Sea; common mainly along the North African coasts, from Gibraltar to Libya, off the coasts of Spain and France, around Sicily and Sardinia, in the Bosphorus and the Black Sea; also widely distributed in the Atlantic Ocean, extending as far north as Newfoundland, northern coasts of the USSR, Norway, Lofoten Islands and Iceland.



A pelagic, very fast swimming species known to effect transoceanic migrations; the young generally form schools, sometimes together with other scombroid species of similar size; immature specimens are found in warm waters only, while adults also enter cold waters in search of food.

Outside the spawning season it is a voracious predator which preys on all kinds of fish, crustaceans and molluscs.

PRESENT FISHING GROUNDS:

Inshore and offshore surface waters.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are collected in Algeria, France (1971: 1 800 tons), Greece, Italy (1971: 3 000 tons), Libya, Malta, Spain, Tunisia, Turkey and Yugoslavia, the catches reported for the area by these countries in 1971 totalling about 8 000 tons.

Caught with trap nets (madragues, almadrabas, tonnarellos) mainly along the coasts of North Africa, Southern Spain, Sicily and Sardinia; in open waters, with trolling lines, floating long-lines and purse seines.

Canning is the most important form of utilization of this highly esteemed fish; it is also marketed fresh.

Fig. 12. (cont'd).

XIPH Xiph 1

1971

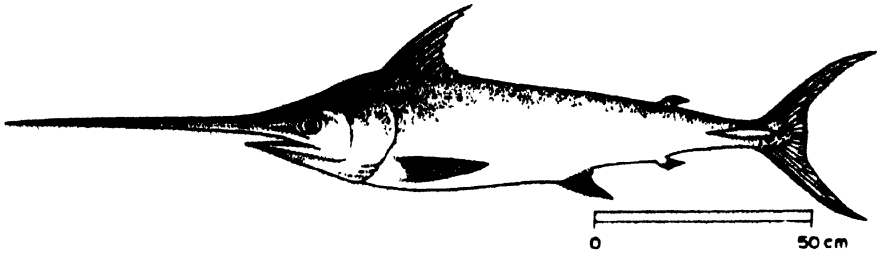
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: XIPHIIDAE

Fishing Area 37
(Medit. and Black Sea)

Xiphias gladius Linnaeus, 1758

SYNONYMS STILL IN USE: None



VERNACULAR NAMES:

FAO - En : Broadbill swordfish
Fr : Espadon
Sp : Pez espada

NATIONAL - ALBN:		ISRL:	Dac haherev	ROMN:	Peste spada
ALGR:	Pissi spada	ITAL:	Pesce spada	SPAN:	Pez espada
BULG:	Mechenosets	LEBN:	Sinka	SYRI:	Kharrayé
CYPR:	Xiphias	LIBY:	Saif	TUNS:	Bou sif
EGYP:	Om sheraa	MALT:	Pixxispad	TURK:	Kilic baligi
FRAN:	Espadon	MONC:	Pisciu spada	USSR:	Mech-riba
GREC:	Xiphiás	MORC:		YUGO:	Igo

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Large and very distinctive fish of rounded body, very robust in front; *the snout ends in a long flattened sword;* young specimens are covered with scales and have single dorsal and anal fins which with growth become divided; *large specimens are scaleless and have a high, but short first portion, and smaller second elements to both fins; pelvic fins absent; a single strong lateral keel on each side of the caudal peduncle;* colour of back and upper sides brownish-black; lower sides and belly light brown.

Other field characters: the shape of the caudal fin is also subject to changes with growth; it is truncate in very young specimens (up to 20 cm in length), later it becomes forked (specimens of about 60 cm in length) and finally semilunar in larger fish; teeth are present only in the young.

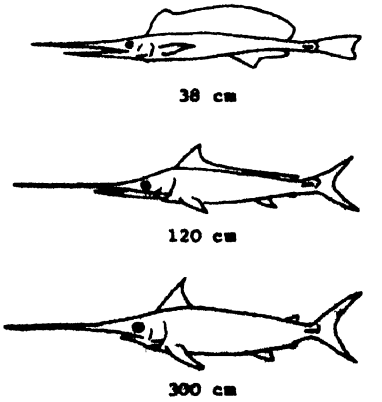


Fig. 13. Identification sheet of Xiphias gladius.

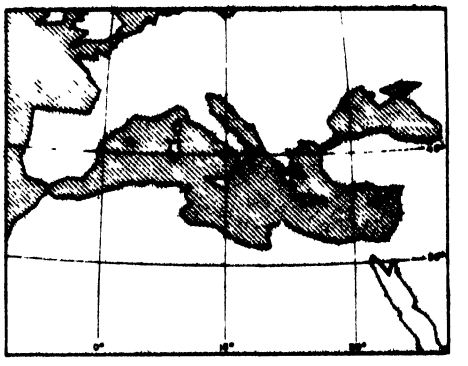
DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

The most similar species occurring in the area belong to the genus *Tetrapturus;* they can be easily distinguished from *X. gladius* by the rather rounded section of their sword and the presence of pelvic fins, as well as of 2 keels on each side of the caudal peduncle.

SIZE:

Maximum: 450 cm; common: 100 to 220 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:



Common throughout the Mediterranean, Black and Azov Seas; of a world-wide distribution in all tropical and temperate oceans; in the eastern Atlantic it occurs from Iceland and the North Sea to a latitude of 45°S.

Highly migratory and very agressive fish, generally not forming schools; found in coastal waters as well as offshore.

Feeds on a wide range of fish, especially schooling species; also on pelagic crustaceans and squids. It is reported to use its sword to kill larger prey.

PRESENT FISHING GROUNDS:

Surface waters, throughout its range.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are collected in Italy (1971: 2 900 tons), Libya, Malta, Spain, Tunisia and Turkey, the total catch reported by these countries for the area in 1971 totalling about 4 500 tons.

Caught mainly with harpoons and floating long-lines (mainly small specimens); in coastal waters it is also taken with fixed nets.

Marketed fresh and frozen.

Fig. 13. (cont'd).

CARAN Trach 1
1971

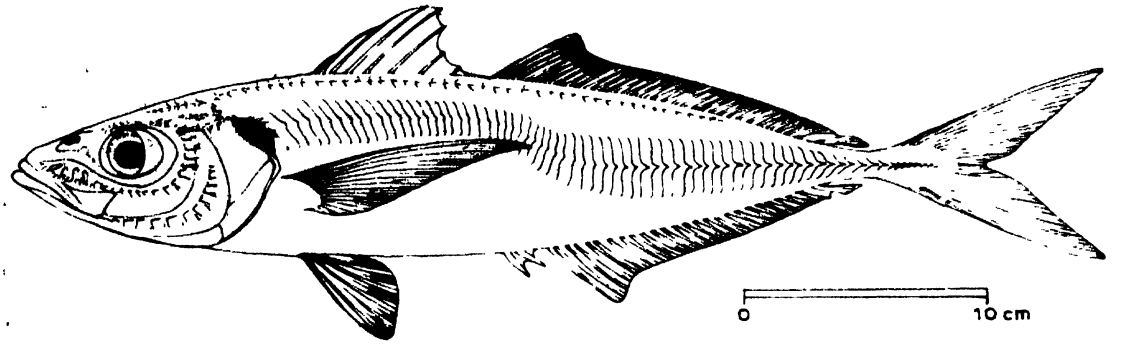
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: CARANGIDAE

Fishing Area 37
(Medit. and Black Sea)

Trachurus trachurus (Linnaeus, 1758)

SYNONYMS STILL IN USE: *Trachurus saurus* Rafinesque, 1810



VERNACULAR NAMES:

FAO - En : Atlantic horse mackerel
Fr : Chinchard
Sp : Jurel

NATIONAL -	ALBN:	Stavrid	ISRL:	Trakhon gedol magen	ROMN:	Stavrid
	ALGR:	Chrel	ITAL:	Suro	SPAN:	Jurel
	BULG:	Okeanski safrid	LEBN:	Assaifar	SYRI:	Esfer
	CYPR:	Safridi	LIBY:	Sauro	TUNS:	Shourou
	EGYP:	Shakhoura	MALT:	Sawrella kahla	TURK:	Istavrit
	FRAN:	Chinchard	MONC:	Cagnassun	USSR:	Stavrida
	GREC:	Savridi	MORC:	Chrene	YUGO:	Snjur

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Two dorsal fins, the first with spines connected by a membrane and higher than the second; two spines connected by a membrane in front of the anal fin; *a complete row of bony scutes along the lateral line, showing a conspicuous slope above the anal spines;* an accessory *upper branch of the lateral line* runs close to the dorsal profile starting from the nape and *extending backwards beyond the middle of the second dorsal fin;* back greyish-green, sides silvery with a metallic-violet gleam, belly white.

Other field characters: a procumbent spine hidden under the skin in front of the first dorsal fin; 69 to 79 scutes along the lateral line.

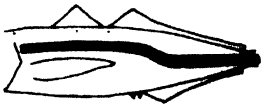
DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

T. mediterraneus: differs from *T. trachurus* by a shorter upper branch of the lateral line which ends at the beginning of the second dorsal fin, and by the smaller size of the scutes.

T. mediterraneus

Fig. 14. Identification sheet of Trachurus.

T. picturatus: differs from *T. trachurus* by the larger number of scutes (98-108) along the lateral line and by the position of the slope farther back than the anal spines.

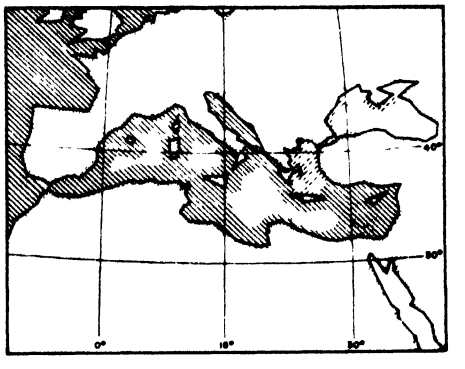


T. picturatus

SIZE:

Maximum: 50 cm; common: about 30 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:



Common in most parts of the Mediterranean, less common in the Aegean Sea, the Sea of Marmara and the Bosphorus; only occasionally recorded for the Black Sea (coasts of Romania and Crimean Peninsula; occurs also in the eastern Atlantic from Iceland and Trondheim Fjord to the Cape Verde islands. Two subspecies are known from other areas: *T. trachurus capensis* (South Africa from Angola to Cape of Good Hope and Delagoa Bay) and *T. trachurus macculloohi* (South Australia).

Pelagic and migratory fish living in rather large shoals, in coastal waters during summer and in deeper waters (down to 500 m) during winter.

Feeds on planktonic crustaceans, fish larvae and young fishes.

PRESENT FISHING GROUNDS:

Continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Only Bulgaria (1970: 700 tons) and Turkey (1970: 7 500 tons) report separate statistics for this species. Statistics are also reported for the general category *Trachurus* spp. which includes both *T. trachurus* (unspecified) and *T. mediterraneus*. The countries reporting on the second category are Algeria (1970: 1 500 tons), France, Greece (1968: 2 900 tons), Italy (1970: 7 800 tons), Malta, Morocco, Romania, Spain (1970: 9 500 tons), Tunisia (1970: 1 400 tons), Turkey (1970: 2 200 tons), USSR, and Yugoslavia with an estimated total for this category of 28 400 tons for 1970. The estimated total of *Trachurus* spp., whether specified or not for 1970, is 36 600 tons.

Fished with trawls and purse seines (using light); occasionally also with longlines and trap nets.

Marketed mainly fresh; also frozen, salted, canned as fillets in oil, and sometimes smoked.

Fig. 14. (cont'd).

CLUP Sardi 1
1971

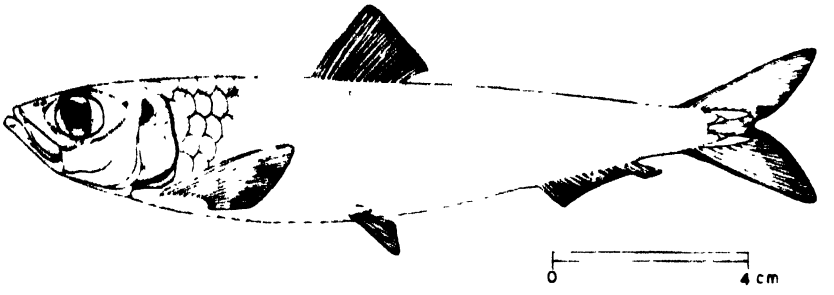
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: CLUPEIDAE

Fishing Area 37
(Medit. and Black Sea)

Sardina pilchardus (Walbaum, 1792)

SYNONYMS STILL IN USE: *Clupea pilchardus* Walbaum, 1792
Sardina pilchardus sardina (Walbaum, 1792)



VERNACULAR NAMES:

FAO - En : European pilchard
Fr : Sardine européenne
Sp : Sardina europea

NATIONAL - ALBN:	Sardelë	ISRL:	Sardin zefoni	ROUM:	Sardea
ALGR:	Sardin'	ITAL:	Sardina	SPAN:	Sardina
BULG:	Sardina.	LEBN:	Sardine mabroum	SYRI:	Sardîn
CYPR:	Sardella	LIBY:	Sărdin mabrum	TUNS:	Sardina
EGYP:	Sardina	MALT:	Sardina kahla	TURK:	Sardalya
FRAN:	Sardine	MONC:	Sardina	USSR:	Sardina
GREC:	Sardélla	MORC:	Sardina	YUGO:	Srdjela

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Body rather rounded, oval in cross section; *belly not sharply keeled in the mid-line, but a shallow ridge runs from the throat to the vent; dorsal fin originates in front of the level of the pelvic fin base; gill cover strongly marked with pronounced radiating ridges; upper jaw not notched in the mid-line;* lower jaw ends in front of the hind margin of the eye; colour of the back greenish, occasionally olive, sides golden, shading to silvery-white ventrally; *a row of faint dark spots along the sides.*

Other field characters: silvery, large, fragile scales which do not extend to the head; no lateral line visible on the sides of the body; the last two rays of the anal fin are longer and broader than the preceding rays; an elongate modified scale is present on both lobes of the caudal fin.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

Sardinella aurita and *S. maderensis:* differ from *S. pilchardus* by the absence of radiating ridges on the gill cover and of dark spots along the sides of the body.

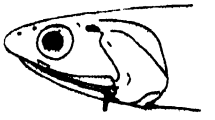
Fig. 15. Identification sheet of Sardina.

Sprattus sprattus: differs from *S. pilchardus* by the sharply keeled belly, with a distinct line of spiny scales running from the throat to the vent, the absence of elongate modified scales on each caudal fin lobe, the position of the dorsal fin, which originates slightly behind the level of the pelvic fin origin and the absence of enlarged rays in the anal fin.

S. sprattus

Alosa alosa and *Alosa fallax nilotica*: differ from *S. pilchardus* by the presence of a distinct notch in the mid-line of the upper jaw and by the absence of enlarged rays in the anal fin.

Engraulis encrasicolus: differs from *S. pilchardus* by the prominent snout and the long upper jaw, the mouth extending well past the eye.

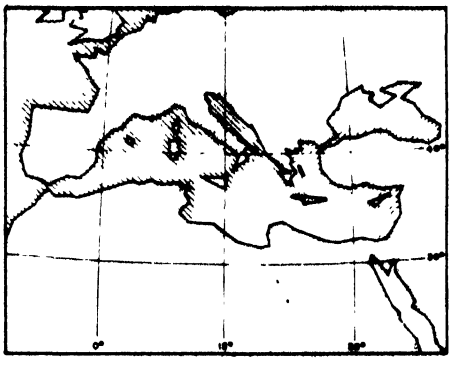


E. encrasicolus

SIZE:

Maximum: 22 cm in the Mediterranean, 17 cm in the Black Sea and 25 cm in the Atlantic; common: 10 to 15 cm in the Mediterranean, 6 to 8 cm in the Black Sea.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:



Common in the western Mediterranean, and in the Adriatic Sea; rare in the eastern Mediterranean, the Sea of Marmara and the Black Sea; also occurs in the eastern Atlantic from Cape Blanc to the Dogger Bank in the North Sea.

A pelagic and migratory fish, forming shoals at shallow depths (15 to 35 m at night and 25 to 55 m by day) in coastal waters over the continental shelf.

Feeds on small phyto-and zooplankton organisms.

PRESENT FISHING GROUNDS:

Coastal waters over the continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are collected in Algeria (1970: 17 000 tons), Egypt, France (1970: 23 000 tons), Greece, Italy (1970: 44 000 tons), Malta, Morocco, Spain (1970: 32 000 tons), Tunisia, Turkey and Yugoslavia (1970: 11 000 tons), the catches recorded for 1970 in the GFCM area by these countries totalling 157 000 tons. However, unidentified quantities of *S. pilchardus* may be included in larger statistical categories by other countries.

Caught with purse seines and lamparas (light fishing), gill nets, beach seines, trap nets and occasionally high opening bottom trawls (French Mediterranean coast).

Canning in oil or sometimes in tomato sauce is the commercially most important form of utilisation in the Mediterranean; also, a rather important part of the landings is pickle-salted, or marketed fresh.

Fig. 15. (cont'd).

1971

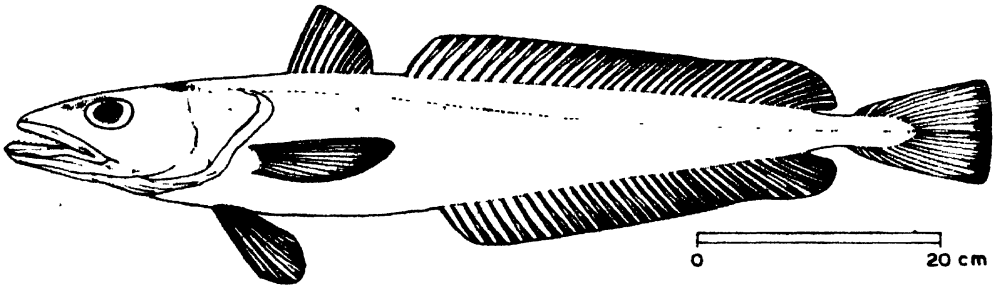
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: GADIDAE (MERLUCCIDAE)

Fishing Area 37
(Medit. and Black Sea)

Merluccius merluccius (Linnaeus, 1758)

SYNONYMS STILL IN USE: *Merluccius vulgaris* Fleming, 1828
Merluccius merluccius mediterraneus Cadenat, 1950



VERNACULAR NAMES:

FAO - En : European hake
Fr : Merlu européen
Sp : Merluza europea

NATIONAL - ALBN:		ISRL:	Zeev hayyam	ROMN:	
ALGR:	Mernûz	ITAL:	Nasello	SPAN:	Merluza
BULG:	Merluza	LEBN:	Armout	SYRI:	
CYPR:	Baccàliaos	LIBY:	Nazalli	TUNS:	Nazalli
EGYP:	Nazelli	MALT:	Merluzz	TURK:	Berlâm
FRAN:	Merlu	MONC:	Naselu	USSR:	Merluza belaia
GREC:	Bacaliáros	MORC:	Lcola	YUGO:	Oslić

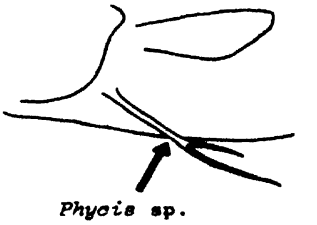
DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Body elongate, with *two dorsal fins, the first higher than the second; the second dorsal and the single anal fin are long and similar in shape with a shallow notch in their outline towards the end;* mouth large extending to the middle of the eye; *no barbles below the chin;* the colour is slate grey on the back, lighter on the sides and white on the belly.

Other field characters: lateral line straight and continuous; lower jaw prominent; large hinged teeth on both jaws; no spiny rays in the fins.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

Phycis blennioides and *Phycis phycis:* differ from *M. merluccius* by their peculiar pelvic fins which have the appearance of a single long branched ray, by the straight outline of their second dorsal and anal fins and the presence of a barbel below the chin.



Phycis sp.

Fig. 16. Identification sheet of Merluccius merluccius.

Molva molva and *Molva elongata:* differ from *M. merluccius* by their long and narrow, almost eel-like body and their short pectoral fins.

Gaidropsarus species: differ from *M. merluccius* by their small and peculiar first dorsal fin which is formed of a series of very short, hair-like rays set in a groove, preceded by one longer ray.

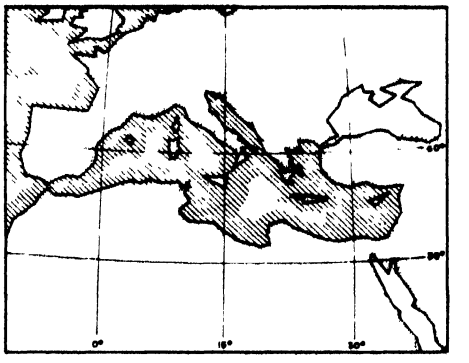
Gaidropsarus sp.

SIZE:

Maximum: 90 cm; common: 15 to 35 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Common throughout the Mediterranean; in the Black Sea it has only been recorded for the south-eastern coast; also common in the eastern Atlantic from Iceland and the Lofoten Islands to Morocco, rare off Mauritania and Senegal.



Usually found at depths between 100 and 400 m, closer to the bottom during day-time, but may occur within a wider depth range, from 30 to 700 m.

Feeds predominantly on crustaceans, also on anchovies, sardines and gadoid species.

PRESENT FISHING GROUNDS:

Offshore waters between 150 and 400 m depth.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are reported by Algeria, Cyprus, France, Greece, Israel, Italy (1970: 8 800 tons), Malta, Spain (1970: 4 600 tons), Turkey and Yugoslavia, the catches in the area reported for 1970 totalling 17 800 tons.

Caught mainly with bottom trawls, long-lines and bottom-set gill nets.

Marketed mainly fresh; recently it is also being frozen, whole or in the form of fillets or steaks; small quantities are salt-dried or smoked.

Fig. 16. (cont'd).

GADI Phyc 1
1971

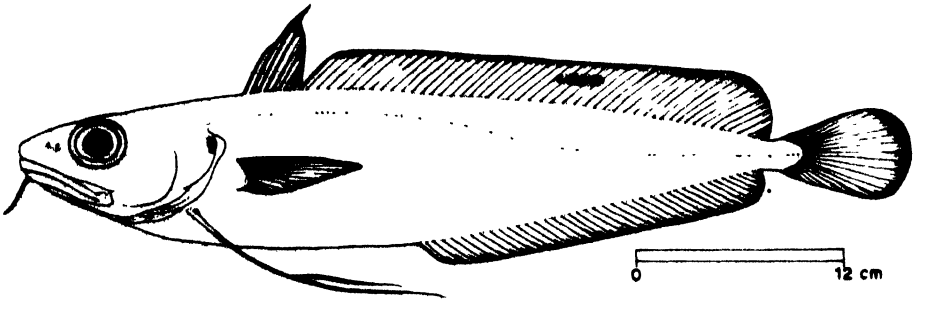
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: GADIDAE

Fishing Area 37
(Medit. and Black Sea)

Phycis blennioides (Brünnich, 1768)

SYNONYMS STILL IN USE: None



VERNACULAR NAMES:

FAO - En : Greater forkbeard
Fr : Motelle de roche
Sp : Brótola de fango

NATIONAL -	ALBN:		ISRL:		ROMN:		
	ALGR:		ITAL:	Musdea bianca	SPAN:	Brótola de fango	
	BULG:		LEBN:	Samak gomok	SYRI:	Kharraye mouassata	
	CYPR:	Malactos	LIBY:		TUNS:		
	EGYP:		MALT:	Lipp abjad	TURK:	Gelincik	
	FRAN:	Motelle blanche	MONC:		USSR:		
	GREC:		MORC:		YUGO:	Tabinja bjelica	

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

Characterised by its *peculiar pelvic fins which have the appearance of a single, long, branched ray extending well past the origin of the anal fin; two dorsal and one anal fins,* the first dorsal very small and of triangular shape with an *elongate third ray, at least twice the height of the second dorsal fin,* the latter long and uniform in height, similar to the anal fin; colour in general brownish to pale pink; dorsal, anal and caudal fins black edged; usually a black spot on the second dorsal fin.

Other field characters: scales relatively large and easily detached, eyes large, lateral line curved anteriorly; no spiny rays in the fins.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:

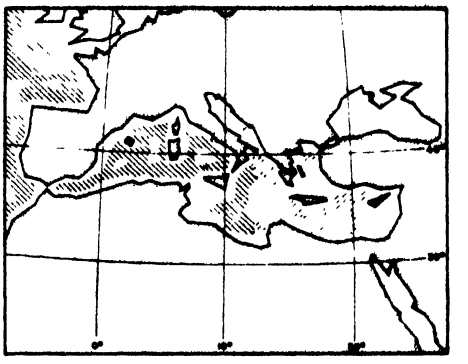
Phycis phycis: differs from *P. blennioides* by the shorter pelvic fin extending only to the origin of the anal fin, the higher body, the lack of elongate rays in the first dorsal fin, the absence of a black spot on the second dorsal, and the dark brown or purplish colour.

All other gadoid species differ from *P. blennioides* by not having modified, thread-like pelvic fins.

Fig. 17. Identification sheet of Phycis blennioides.

SIZE:

Maximum: 75 cm; common: about 30 cm.



GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:

Common in the western Mediterranean, rare in the Adriatic and the eastern basin, absent from the Black Sea; also occurs in the eastern Atlantic from Iceland and Norway to Morocco.

Found in the proximity of the sea bottom over muddy grounds, being most common at depths between 150 and 300 m, but occurring occasionally down to 800 m.

Feeds predominantly on crustaceans and fishes.

PRESENT FISHING GROUNDS:

Muddy bottoms of the deeper continental shelf areas and the slope.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are reported by Spain only (1970: 500 tons); probably the total Mediterranean catches are much higher, as unidentified quantities of this species may be included in larger statistical categories in other countries.

Fished mainly with bottom trawls and long-lines.

Marketed fresh only; not highly esteemed.

Fig. 17. (cont'd).

1971

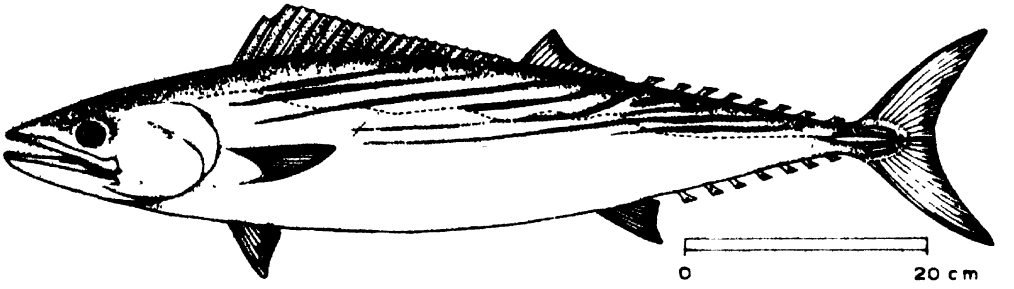
FAO SPECIES IDENTIFICATION SHEETS

FAMILY: SCOMBRIDAE

Fishing Area 37
(Medit. and Black Sea)

Sarda sarda (Bloch, 1793)

SYNONYMS STILL IN USE: None



VERNACULAR NAMES:

FAO - En : Atlantic bonito
Fr : Bonite à dos rayé
Sp : Bonito

NATIONAL -	ALBN:	Palamiti	ISRL:	Sarda	ROMN:	Palamida
	ALGR:	Bonite	ITAL:	Palamita	SPAN:	Bonito
	BULG:	Palamud	LEBN:	Ghazel	SYRI:	Balmida
	CYPR:	Palamida	LIBY:	Balamit	TUNS:	Balamit
	EGYP:	Balamita	MALT:	Plamtu	TURK:	Palamut
	FRAN:	Pélamide (Bonite à dos rayé)	MONC:	Paramida a schina rigà	USSR:	Palamida
			MORC:	Bonite	YUGO:	Polanda
	GREC:	Palamida				

DISTINCTIVE CHARACTERS AND DIAGNOSIS:

A small, relatively narrow-bodied tuna; finlets present behind the second dorsal and the anal fins; *dorsal fins close together, the first (spiny) dorsal fin very long and straight or only slightly concave in outline; mouth rather wide, the upper jaw reaching to the hind edge of the eye or beyond;* pectoral fins short; *lateral line conspicuously wavy; two flaps* (interpelvic process) *between the pelvic fins; body entirely covered with scales which are minute, except on the well developed corselet* (area behind the head and around the pectoral fins covered with larger and thicker scales); on each side of the slender caudal peduncle, a well developed lateral keel between two small keels located at the bases of the caudal fin lobes; colour of the back and upper sides steel-blue, with *5 to 11 dark oblique stripes* running forward and downward; lower sides and belly silvery.

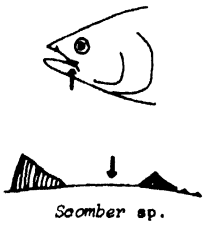
Other field characters: 7 to 10 dorsal, and 6 to 8 anal finlets.

interpelvic process

S. sarda

Fig. 18. Identification sheet of Sarda sarda.

DISTINCTION FROM MOST SIMILAR SPECIES OCCURRING IN THE AREA:



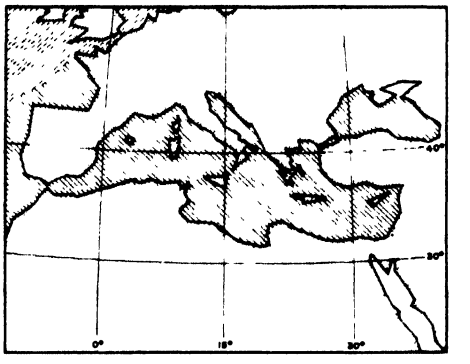
Scomber sp.

All other scombroid species occurring in the area have a shorter upper jaw which never reaches up to the hind edge of the eye and a shorter, clearly concave first dorsal fin. Many of the tuna species are considerably larger and all have a different colour pattern; the mackerels *(Scomber)* and frigate mackerels *(Auxis)* have widely separated dorsal fins (interspace at least equal to the length of the first dorsal fin base).

SIZE:

Maximum: 80 cm (85 cm in the Atlantic); common: 30 to 50 cm.

GEOGRAPHICAL DISTRIBUTION AND BEHAVIOUR:



Common throughout the Mediterranean, in the Black Sea and in the tropical and subtropical waters of the Atlantic; present also in higher Atlantic latitudes, up to the coasts of Scandinavia and Ireland.

Pelagic migratory species often schooling near the surface in inshore waters.

Feeds mostly on fishes, particularly small clupeoids, gadoids and mackerels.

PRESENT FISHING GROUNDS:

Surface waters, mainly over the continental shelf.

CATCHES, MAIN FISHING GEAR AND PRINCIPAL FORMS OF UTILIZATION:

Separate statistics for this species are collected in Algeria, Bulgaria, Greece (1971: 2 000 tons), Italy (1971: 1 000 tons), Malta, Morocco, Romania, Spain, Tunisia, Turkey (1971: 20 000 tons), USSR and Yugoslavia, the catches reported for 1971 within the area by these countries totalling about 25 000 tons. However, unidentified quantities of this species may be included in some countries in larger statistical categories.

In coastal waters it is caught mostly with gill nets and purse seines, while trolling lines are more often used offshore.

Marketed mainly fresh and canned.

Fig. 18. (cont'd).

Molluscs with only one shell, never in the form of a spiral, cup- or dish-shaped

1) shell with one or more holes or a slit at the margins of the shell — 2

– shell without holes or slit — 3

2) large ear-shaped shell with a series of holes along the shell margins; apex asymmetrical — Haliotidae

– shell with a small hole in the apex or a slit in the shell margins — Fissurellidae: Emarginula, Diodora, Scissurella sp.

3) shell circular or elliptical with radial ribs, apex more or less central — 4

– small shell with apex slightly bent to one side of the shell, — Acmaea sp.

4) shell diameter about 3 to 5 times shell height — 5

– shell diameter about 2 times shell height — 6

5) shell diameter about 3 to 4 times shell height, radial ribs of equal strength and elevation, shell white-reddish with black spots — P. lusitanica (Gmel.) (= P. rustica (L.))

– radial ribs of the shell of different strength and elevation, shell margins smooth to very regular. Species represented in many different variations; very common in the Mediterranean Sea; up to 45 mm in Ø, shell of bluish color, thinner than P. vulgata — P. coerulea (L.)

6) apex of the shell wart-shaped, shell whitish, lower part with brown stripes or a small brown ring, shell margin regular — P. mamillaris (Lam.)

– without wart-shaped apex, shell margin smooth, very common in English Channel, Atlantic Ocean, North Sea but only present in the very western part of the Mediterranean; up to 55 mm in Ø — P. vulgata (L.)

Fig. 19 Key to distinguish Patella species from other gastropods

3. SAMPLING SITES

Pollution will be primarily monitored in the coastal areas of the Mediterranean proper (Black Sea is excluded) with the exception of Hg and Cd which will also be determined in pelagic tuna or swordfish.

The geographic distribution of sampling areas along the coast should be guided by the presence of major pollution sources such as rivers, towns and industrial complexes which discharge into the sea. Since it is the aim of these pilot studies to assess the general concentration pattern of certain potential pollutants, samples should not be taken in the direct vicinity of pollution sources such as pipelines from industrial plants, sewage outfalls from towns and villages, nor in ports or in small estuaries of highly polluted rivers but from areas which are representative for the general conditions of a certain coastline. In addition, highly polluted areas may be sampled in order to assess the existing maximum pollution. Existing or planned marine parks should be used as reference areas, whenever possible.

Sampling sites must be clearly identified on a map and the general characteristics of the site described. For the samples taken at sea the position should be given in coordinates.

As a wide variability in the concentrations of a pollutant in the specimens of a certain species collected in one sampling area can be anticipated, the "minimum programme" calls for the analysis of composite samples in which samples taken from different individuals of a species are pooled. This requires that sampling locations are selected according to a plan (see section 7) which makes sure that the variability encountered in the sampling area is really represented in the composite samples. The analysis of pooled samples will only supply data which are equivalent to mean values without an indication of the variability between individual organisms. In order to establish statistically significant differences between composite samples the variability between at least some additional single species-samples must be determined.

In selecting sites, besides easy access, the abundance of the species must be considered, so that enough material will be available from the same site during the entire pilot project without depleting the resource.

4. SAMPLING PROCEDURES

Several different sampling procedures will be needed to obtain the required samples. Wild mussels are collected by detaching them from rocks and other substrate, cultured mussel will be supplied from mussel gardens. Shore crabs, shrimp and mullet can be obtained from bottom trawling. The mullet can also be obtained with beach seine and gill nets. The pelagic tuna is caught with trolling lines and floating long lines and purse seines in open waters. In certain areas special trap nets (tonnara, madragues, almadrabas, tonnarellos, southern Italy, southern Spain, and north Africa) are employed. The swordfish is caught with floating long lines or with harpoons from specialized boats possessing a very high lookout mast. Sometimes they are also caught with fixed nets in coastal waters.

Except for the cases in which the participating centre has its own research vessel, the most efficient way to obtain the samples is with the help of a professional fisherman. It is not advisable to buy the specimen needed for the samples on a fish market, since often the fish vendor cannot guarantee that the fish sold really comes from the sampling area selected by the participating centre. With a commercial fishing vessel and crew at disposal one can give the necessary instructions so that the samples are actually taken at the different locations of the sampling area. It is, however, necessary that the person responsible for the sample collection at least initially accompanies the fishermen during the seasonal sampling trip, in order to introduce the fishermen to the special precautions to be taken and to supervise the sampling procedure.

Sample preparation should possibly be postponed until after shipment of the specimens to the participating centre or to a laboratory which is located near to the sampling area. This will reduce contamination.

The transport of mussels, shrimps, crabs and fish which are caught near the laboratory will not present any special transport and storage problems. Mussels, especially when collected from the intertidal zone, will be able to survive aerial exposure for more than one day. Mussels submerged in a bucket will open their valves and start pumping water and excreting waste products, while during aerial exposure their metabolic rate is greatly reduced (Coleman and Trueman, 1971). It is, therefore, advisable to keep the mussels in air, and moisten them with sea water collected from the sampling area so that they do not excrete their waste products into the water and contaminate other mussels. A grid on the bottom of the bucket etc. may help to prevent the mussels from being submerged. If longer transportation is necessary the mussels for metal analyses are to be stored in plastic buckets or bags placed into thermoisolated boxes. Metal buckets, glass, porcelain or unchipped enamel containers (if necessary in thermoisolated boxes) are needed for the temporary storage of mussels for the Chl-HC analysis. Great care is again necessary to avoid accumulation of sea water in the bottom of the containers.

Dead fish, crabs or shrimps can be kept in thermoisolated boxes in plastic materials for metal analysis and in metal, enamel, porcelain or glass containers for Chl-HC analysis. If hard glass (Pyrex or similar) containers are used the fish and shellfish samples both for metal and Chl-HC analyses can be kept together which may be an advantage even though glass containers are much more fragile than plastic or metal containers.

One of the major difficulties in the proposed analyses will be to minimize the contamination of the sample during sampling, preparation, etc. The sources of contamination are many: paint chips, grease from winches and cables, engine cooling water, sea water or rain-water runoff from the ships deck, water draining from the wet ice used to cool the samples (PCB contamination), air-born particles from the engine exhaust and last but not least, the hands of sampling operators and the sample containers themselves.

In order to avoid these hazards special precautions are necessary. For example, samples should not be hauled aboard on the side where cooling water of the engine is discharged. During sampling the ship should be positioned in such a way that the engine exhausts do not fall on deck. In order to avoid sample contamination from wet ice used for cooling, the samples can be placed into watertight containers. Since most samples will be prepared later during the sample preparation in such a way that only internal parts are used it is not necessary that the persons who handle the samples wear gloves but the sample operator should clean his hands carefully with detergents and clean sea water.

For cleaning of instruments, plastic materials and aluminum foils and general precautions during the handling of samples see sections 5.1.1 and 5.2.1 .

If samples have to be taken on vessels which do not return daily, one has either the choice of preparing the samples aboard or freeze the whole specimen and prepare the sample later on a partially thawed specimen. Dissecting only partially thawed specimens serves to avoid losing substances contained in the "drip". It is advisable to collect a sufficient number of spare specimens so that, especially at the start of the pilot project when the personnel is not yet experienced with the procedure, extra specimens are available for trying out both the sample preparation and the chemical analyses. In order to save time and to process the sample specimens without delay specimens may be obtained from the general fish market for these trial runs.

Since length measuring boards of wood or plastic are not suitable for length determinations of the specimen to be used for micro-analyses, a Pyrex dish can easily be prepared with a cm scaled band attached under the glass bottom. A normal tailor's band will be sufficient

but, cm scaled adhesive tape is now available from chemical supply firms. Otherwise a scale can be incised into the glass with a diamond pen. Using glass has the advantage that the dish prepared for length measurements can be used for both types of micro-analyses.

By no means should live marine organisms sampled for the monitoring programme be kept in a sea water circulation system, since most, if not all, such systems have much higher metal concentrations than natural sea water and also other contamination hazards are difficult to exclude.

Sampling should be carried out according to a sampling plan which possibly supplies, besides mean values from the analysis of composite samples, also information on the precision and random error of the analytical procedure and the biological variability between specimens of the same species caused by intrinsic and environmental factors (see section 7).

If it is absolutely impossible to obtain one of the species of first priority in the sampling a closely related species or one listed under the species of secondary priority should be collected. One must, however, keep in mind that this will make the comparison of results from different institutes very difficult.

In order to allow a secure identification of the samples collected all samples should contain at least the following :

i) name of species sampled
ii) name of the laboratory
iii) name of the sample collector
iv) date and location of sampling
v) code number of the sample

4.1 Sampling of mussels

Samples of Mytilus will serve both for analyses of metals and Chl-HCs. Therefore, two partly different sampling procedures are necessary.

For metal analyses:

Equipment needed:

a) Plastic buckets or thermoisolated boxes containing plastic grids in order to avoid the mussels being submerged in the sea water used to keep them moist. Camping equipment may be adapted for this use.

b) A plastic bucket for sea water to keep the mussels moist.

c) A scraper or similar device (see Fig. 20) or diver's knife of stainless steel.

e) Use of a small rowing boat.

Presampling preparations:

Clean scraper, diver's knife, thermoisolated boxes and buckets with detergents and rinse with tap water or clean sea water from the sampling zone.

For analyses of Chl-HCs:

Equipment needed:

a) Buckets or thermoisolated boxes of non-rusting metal containing a metal grid in in the bottom.

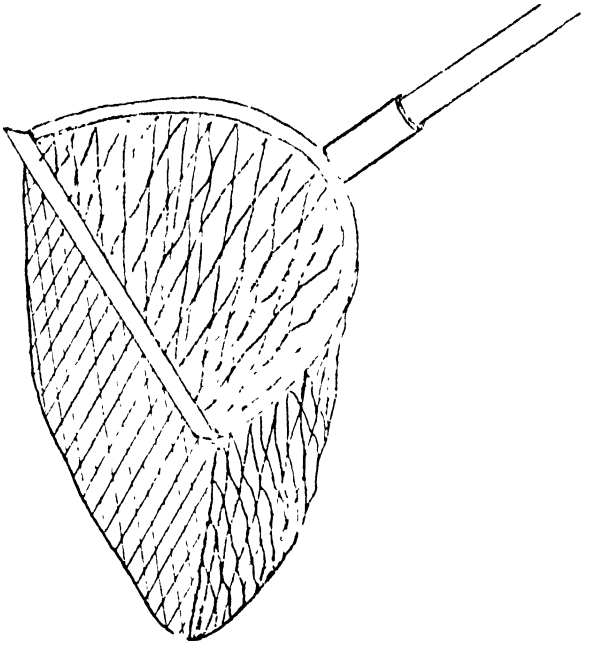


Fig. 20 A scraper for collecting mussels.

b) Metal buckets for sea water to keep the mussels moist.

c) A scraper (see Fig. 20) or similar device or diver's knife of stainless steel.

d) Use of a small rowing boat.

Presampling preparation:

Clean scraper, diver's knife, thermoisolated boxes and buckets with detergents and rinse with sea water from the sampling zone.

Sample characteristics:

Shell length: 4-5 cm

Minimum number of mussels for composite sample: 10

Approximate yield from one mussel: 1 g FW

Mussels attached to metal pipes, ships hulls or other hold-fasts which may have contaminated the mussels either with metal or Chl-HCs must not be included in the samples. If wild mussels of the desired sizes are not available, smaller sizes or mussels from mussel gardens may be sampled. If several mussel gardens are available for sampling the garden in the apparently cleanest waters should be selected. The one in the apparently most polluted waters may be sampled for assessing maximum contamination.

Since a great variability in concentration of pollutants in mussels from different sampling stations of an area is to be expected, in a first approach mussels from different locations of the site and from different depths should be pooled for the composite sample. A composite sample should contain the edible part of at least 10 individual mussels, free of the byssus and the pallial fluid. Additional mussels for the estimation of the variability of pollutant concentration should be sampled whenever possible (see section 7).

The actual sampling of wild mussels may be carried out from the shore, but it is probably more efficent and convenient to sample from a small rowing boat with a scraper fixed to a long stick. One person is needed to control the boat with the oars while another is scraping the mussels from the supporting rocks or substrate. The scraper should be made of stainless steel and the collection net of nylon. One other way would be through the assistance of a skin diver who collects the mussels by removing them with a good stainless steel diver's knife and collects them in a nylon net.

If samples are taken from a mussel garden culture, the mussels are to be collected from various depths and from different positions within the mussel culture.

4.2 Sampling shrimps for analyses of chlorinated hydrocarbons

Shrimps (Parapenaeus longirostris) are recommended for Chl-HC analysis.

Equipment needed:

a) Thermoisolated boxes with metal, glass or porcelain insert (camping equipment).

b) Al-foil or all metal or glass container (household ware, camping equipment) of sufficient size for temporary storage and transport of samples.

c) A Pyrex dish prepared as length measuring device (see page 40).

d) Use of a vessel with bottom trawl nets.

e) During warm periods, refrigerator (+1 to +4°C) or wet ice for cooling. Deep freezer, if sampling trip takes longer than 48 hours.

Presampling preparation:

Clean thermoisolated boxes, length measuring device, Al-foil or metal (stainless steel) containers with 95% ethanol of high purity.

Sample characteristics:

Length of shrimps: 8–10 cm

Minimum number of shrimps for composite sample: 10

Approximate yield per shrimp: 5–8 g FW

At least 10 shrimps are necessary for each composite sample. The shrimps are caught with bottom trawl nets. As soon as the catch comes aboard shrimps of 8–10 cm length are selected and groups of 12 shrimps are wrapped with heavy duty Al-foil or placed into separate metal containers. If the extremities of the shrimps puncture the Al-foil an additional foil will be needed. The sample identification note (p. 42) is then placed on the Al-wrapped package and the whole is rewrapped with another Al-foil. If the shrimps are placed in metal containers, the note is attached to the box or the information is written directly on the container. The Al package or the metal container is then stored in a refrigerator (+1 to +4°C) or in thermoisolated boxes kept cool with wet ice. If the vessel does not return into port within 12 hours after the last sampling the samples have to be deep-frozen. If a clean cabin is available aboard, sample preparation can be carried out aboard, otherwise it is advisable to handle the sample as little as possible aboard in order to reduce contamination hazards.

Sample preparation aboard:

Sample preparation aboard requires that the shrimps length is determined on a length measuring device. The length is to be recorded from the rostrum to the uropod (Fig. 3) and the individual shrimps are weighed to the nearest 0.1 g. The abdomen is then separated from

the carapace and from the tail (telson and uropod) with a metal knife, taking care that all viscera are discarded together with the carapace.

If there is the slightest doubt that the sample preparation aboard might result in contamination of the sample, the sample preparation should be postponed until a clean laboratory room is available and instead, the shrimps should be deep frozen (less than $-18^{\circ}C$).

If necessary, a few shrimps should be preserved, either deep-frozen or in formaline or alcohol, for a species identification check (see section 2.1).

4.3 Sampling of shore crabs for analyses of chlorinated hydrocarbons

The expert consultation recommended crab samples (Carcinus mediterraneus) for Chl-HCs analyses as substitutes for P. longirostris in those areas where the shrimps are not available.

Equipment necessary: same as for shrimps.

Presampling preparation: same as for shrimps.

Sample characteristics:

Length of crab carapace: 2-4 cm

Minimum number of crabs for composite sample: 10

Yield of mussel from 2 chelae: 0.2-0.5 g FW

The chelae of at least 10 crabs (carapace length 2-4 cm) are needed for a composite sample. Crab samples can be obtained with bottom trawls, beach seine nets and with pots. On the basis of local experience the best method shall be selected.

The sampling scheme calls for only the chelae to be analysed. In order to reduce the volume of the sample and minimize contamination hazards the chelae should be separated as soon as possible after sampling and preserved as described for shrimps. If the chaelae cannot be prepared for chemical analyses within 13 hours they must be deep frozen (less than $-18^{\circ}C$). Include the sample identification note (page 41).

After separating the chelae the length of the carapace is determined and if necessary a few crabs complete with chelae are preserved for a species identification check (see section 2.1).

4.4 Sampling of Mullus barbatus

M. barbatus will serve for the monitoring of both metals and Chl-HCs. In order to avoid contamination two different sampling procedures, one for metal and one for Chl-HCs, must be considered.

For Metal Analysis

Equipment needed:

a) Plastic thermoisolated boxes

b) High density polyethylene bags

c) Plastic length measurement board or a Pyrex dish with cm scale (see page 40).

d) Use of a bottom trawler or a fishing boat with seine nets

e) During warm periods, refrigerator (+1 to +4°C) or wet ice for cooling. Deep freezer, if sampling trips take more that 12 hours.

Presampling preparation:

Clean thermoisolated boxes, high density polyethylene bags, and length measuring device with detergents and rinse with distilled water or clean sea water from the sampling zone.

Sample characteristics:

Length of fish: 10–15 cm

Minimum number of fish in a composite sample: 6

Yield of fillets per fish: 8–15 g FW

Bottom trawl nets are in general used to catch M. barbatus but the mullet can also be obtained with beach seine nets and gill nets. After the catch is taken aboard, mullets of 10–15 cm length, with undamaged skins, are selected. Their approximate length can be determined on a clean dish with a cm scale. They are placed in a precleaned plastic bag after which the air is squeezed out and closed with a knot or thermoseal. The bag is then placed along with the sample identification note (page 42) into a second plastic bag and closed in the same way as the first bag. If necessary the sample is then placed in a refrigerator (+1 to +4°C) or in a thermoisolated box which is cooled with wet ice. Care must be taken that no water from the wet ice enters the box to avoid contamination.

For the Analysis of Chl-HCs

Equipment needed: same as for shrimps.

Presampling preparation: same as for shrimps.

The sampling steps are the same as described for metals, above, but the fish are packed in precleaned Al-foil, glass containers, etc. instead of plastic bags. Special care must be exercised so that fins and tail will not puncture the Al-foil. The samples are then placed in thermoisolated boxes with metal, glass or similar insert (not plastic). If wet ice is used for cooling it must be kept separate from the fish in order to avoid contamination. Prepare the sample identification note (page 41).

4.5 Sampling of tuna and swordfish

Tuna and swordfish will serve mainly to monitor mercury and cadmium. Since tuna and swordfish are usually large fish of considerable value sampling will, in most cases, be confined to subsampling specimens caught by commercial fishermen. Before selling tuna or swordfish the tail is usually cut off and hence it should be relatively easy to buy cuts near the tail from several specimens without great expense. Every effort should be made to obtain sections of 3 to 4 cm thickness of at least three specimens, and data on weight, length of specimens, sampling area, fishing time and technique.

Equipment needed:

a) High density polyethylene bags.

b) Plastic thermoisolated box for the transport of the subsamples.

c) A ruler with cm scale for measuring the length of the specimens.

Presampling preparation

Clean plastic bags and thermoisolated boxes with detergents and rinse with distilled water.

Sample characteristics:

Possibly a slice of 3 to 4 cm thickness free of skin and bones cut from the tail end of three different specimens.

Sampling procedure:

a) Acquire 3 to 4 cm thick sections of possibly at least three tuna or swordfish from a commercial fisherman and place in individual plastic bags. Supply the bags with the sampling identification note (see page 41).

b) Estimate the fork length (see Fig. 3) of the specimens sampled. Collect information on the weight of the specimens, sampling area, fishing time and technique. Prepare the sample information sheet (Table II).

4.6 Reporting of biological data and sampling procedure

It is of an extreme importance that each sample will be accompanied with the relevant biological data and data on the sampling procedure and the sampling site.

The minimum data necessary are:

a) Species, scientific and local names.

b) Type of analyses

c) Date of collection

d) Area of collection: description of the geographic position on the coast line, coordinates or length from known positions.

e) Name of the person responsible for the sampling, name of the fishing vessel and captain, if applicable.

f) Sampling and/or fishing method used.

g) Storage method used before sample preparation.

h) Presample preparation, storage time and storage method used (i.e. stored in refrigerator, deep frozen, oven dried at 60°C etc.).

i) Length of individual specimens.

j) Weight of individual specimens.

k) Code number of the sample container.

Additional information on general oceanographic data such as water temperature, turbidity, state of the sea, salinity, other complementary analyses on water samples etc. are useful. An example of a "sample information sheet" is given in Table II.

Table II

Example of a sample information sheet

Species	Mullus barbatus
Analyses	Metals
Date	1975-10-17
Area	2 km off coast of Marina di Carrara, -18 m depth, sandy bottom
Name of responsible	A. Secondini, M/V 'S. Gabriele', capt. Guidi
Gear	bottom trawls
Predissecting storage	1 day in refrigerator (circa +4°C)
Final storage	deep frozen in plastic containers

Sample code	Fork length in cm	FW of fish in g	FW of fillets in g	Number of fillets	Weight included in the sample 1/
F 1	12.5	38.8	16.0	2	10.0
	13.5	41.3	14.1	2	10.2
	12.5	34.1	12.9	2	9.9
	11.5	27.4	10.1	2	10.1
	12.5	32.4	10.8	2	10.1
	12.3	34.4	11.1	2	9.8
		Total fillets:	75.0	Total sample :	60.1 g FW
F 2	12.6	35.2	12.2	2	10.4
	14.0	47.3	15.9	2	10.6
	12.7	31.0	10.6	2	10.5
	12.5	33.1	12.7	2	10.4
	13.4	50.1	14.0	2	10.6
	13.2	41.3	12.8	2	10.7
		Total fillets:	78.2	Total sample :	63.2 g FW

1/ Fillets have been cut to about equal size so that the determination of mixed samples is representative for the mean concentration (see section 7.2).

5. SAMPLE PREPARATION

During sample preparation special precautions must be taken to minimize contamination of the sample, but equally important is that during sample preparation no losses of volatile metal organic compounds or Chl-HCs occur. The contamination of the samples is a very serious problem for the microanalyses proposed in the pilot projects. It is, therefore, absolutely necessary to take precautions to avoid any contact with substances which may alter the concentration in the sample. The contamination problem is more serious for sea water samples than for organisms since in the latter the metals and Chl-HCs have higher concentrations than the water and subsamples can be taken from an organism in such a way that the contamination risk is largely excluded.

The losses due to volatilisation during storage can be reduced if the samples are enclosed in hermetically sealed glass or quartz ampoulles with breakoff tips and the ampoulle is introduced directly into analytical apparatus. Since this procedure is quite cumbersome it is suitable only for long term storage.

The next best procedure would be to deep freeze the sample in air tight containers. This would imply that the samples are kept in a frozen state until analysis. This is not difficult to achieve, if the samples are to be transported only over short distances. However, shipping frozen sample by commercial carriers such as railroads and airline present problems, since even samples shipped in dry ice will not stay frozen longer than 24 hours at best. Taking into consideration the uncertainty of many transportation time schedules it is not advisable to ship samples in a frozen state over long distance and with complicated travel schedules. A sample in a sealed glass or quartz ampoulle would here be preferred.

An alternative way would be shipping the samples after freeze drying, although losses of organo-metalic volatile compounds and of Chl-HCs during freeze drying have been reported (La Fleur, 1973, Pillay, 1972). Oven drying at low temperature (60°C) could be a suitable method, if it can be shown that no losses occur.

If the samples are to be analysed within a period of 2 years after sampling in the laboratory which has collected them, deep freezing is the best preservation method since the combustion of the organic matter is easier in fresh or frozen material than in dry tissues. If it can be shown that biological material dried at low temperature will not cause losses; dried materials will have the advantage of simpler storage.

In order to avoid contamination from instruments and other materials which may come in contact with the samples, great care must be exercised in selecting instruments which will minimize contamination. Unfortunately the same instuments and materials cannot be used for the preparation of both the samples destinated for analyses of heavy metals and Chl-HCs.

5.1 Sample preparation for analyses of metals

5.1.1 Selection and cleaning of instruments and material

For metal analyses all handling and sorting gear should be high density polyethylene or teflon (Bertine and La Fleur, 1972). Stainless steel may also be used in the pilot projects, since good stainless steels posses very low concentrations in the heavy metals on the first and second priority lists. However, if it is also planned to use the samples for multi-element analyses of other metals, only high density polyethylene, teflon or other plastic materials of high purity which will not contaminate the sample with inorganic elements and substances, must exclusively be used. Plastics even of high purity can be surface contaminated with heavy metals (see section 6.1) and must be checked after cleaning. (See Appendix A for instructions to make plastic tweezers.)

Zinc plated instruments sometimes sold as "inox" steel must absolutely be avoided. Stainless steels of high corrosion resistance are not magnetic, and can therefore be distinguished from other stainless steels with the use of a magnet.

Only borosilicate glass of a known brand (Pyrex, Duncan, Hylar, Jena 50, etc.) should be used, since many soft glass contains large amounts of lead and other impurities. Instruments, and plastic or glass containers used in handling and storage should be cleaned with detergents and then rinsed with distilled or deionized water. The same procedure can be used to clean the plastic sheets which will serve to cover the working table and other surfaces used during the sample preparation. In addition or in substitution to the detergent 1 N H_2SO_4 or 1 N HNO_3 obtained by diluting concentrated H_2SO_4 or HNO_3 with glass distilled water can also be used.

Distilled water may be substituted by clean sea water, which may be collected at 10 m depth (to avoid the contamination of the vessel), possibly off the continental shelf or where the depth is at least 100 m. This sea water often contains smaller amounts of heavy metals and other impurities than the usual double distilled water employed in most laboratories.

Under no circumstances should commerically available distilled water be used unless the supplier can guarantee that the water is distilled from an all-glass or quartz-still. Commercially distilled water often comes from copper apparatus.

Subsampling of organisms and sample preparation should be carried out in a dust free room. The working tables should be covered with pre-cleaned plastic sheets. Plastic gloves may be used during the operations. However, their use is cumbersome and one has to weigh the reduced contamination hazard against the increased difficulty of handling the sample. If whole organisms are to be used as samples for metal analyses, they should be handled with gloves. On the other hand in the pilot projects the organisms will be subsampled in such a way that the outer surfaces will be discarded. Therefore, if the subsampling operation is carried out correctly, and with clean instruments, there is no need to work with gloves.

The sheets and, if necessary, the gloves are cleaned with detergents or acids (see above) and rinsed with distilled water or clean sea water. Flame resistant Pyrex vessels and jars with their corresponding lids which are obtainable in normal household stores provide very suitable supports during the sample preparation, and are much easier to handle than plastic sheets and bags.

5.1.2 Sample preparation for deep-freezer storage

After the specimens have been sampled they will be treated and placed in plastic bags as described below. For small samples, containers of pure high quality plastics with screw caps or similar are preferred. The following procedure has then to be modified accordingly.

1) Weigh the dry plastic bag (which has been precleaned in detergents and distilled water) to the nearest 0.1 gram on a balance. Note the weight.

2a) Composite sample: cut each specimen-sample starting with the smallest to about the same weight so that each specimen-sample is of the same size. Place the specimen-samples in the bags, weigh the container with its contents and calculate the freshweight (FW).

2b) Single specimen-sample: Place the specimen in the bag and determine the FW.

3) Release the excess air from the bag by rolling it gently over the sample without harming it.

4) Close the plastic bag airtight by heat sealing above the sample or by some other method.

5) Prepare a 'sample identification note' (page 41) by taking the necessary data from the sampling protocol.

6) Place the 'sample identification note' above the heat seal in the remaining part of the plastic bag and heat-seal the plastic bag again.

7) Place the bag into a second bag, press out the excess air as before and heat-seal again.

8) Place the bag containing the sample in a deep freezer which maintains a temperature below -18°C.

Samples should be frozen fast in order to avoid the formation of large ice crystals within the tissue cells of the sample. Large ice crystalls will rupture the cell walls and upon thawing excessive amounts of cellular liquids ("drips") will form. Since this "drip" may contain part of the elements and substances to be analyzed, "drip" formation should be minimized by fast freezing. All "drip" has to be considered as part of the sample.

In order to guarantee fast freezing and not to overload the freezer with fresh material, approximately 1 kg for every 50 litre net volume can be frozen in a normal household deep-freezer every eight hours without excessively raising its internal temperature. It is advisable to bring the freezer to its lowest possible temperature before starting to deep-freeze the samples. Placing fresh materials in direct contact with the freezer plates facilitates heat transfer and accelerate the cooling of the material. Unfrozen materials should never be placed in direct contact with already frozen materials. Even if the material is not thawed, its storage time will be greatly reduced if brought above -18°C. The material awaiting to be deep-frozen should be stored in a normal refrigerator at near freezing temperature (+1 to +4°C).

5.1.3 Sample preparation of Mytilus

The composite sample should contain at least ten soft parts, i.e. the mussel's body without the shell. The holdfast of the mussel (byssus in Fig. 1) is to be eliminated and the pallial fluid, i.e. the fluid in the mantle or pallial cavity, should be discarded. Figure 1, and its accompanying text may be consulted for more details of the mussel's external morphology. For sample preparation two persons are needed. Special attention must be paid to avoid contaminating the soft part by holding the outer part of the shell, while rinsing the soft parts during the sample preparation. Ensure that the rinsing liquid does not come in contact with the outer shell before rinsing the soft part. In order to minimize contamination of the soft part, two different knives and two different tweezers are needed. One knife (No. 1) and one tweezer (No. 1) should be used to scrape the shell in order to eliminate epiphytal and epifaunal growth, etc., and one knife (No. 2) and one tweezer (No. 2) for cutting the two adductor muscles of the mussel and for lifting out the soft part.

Sample characteristics:

a) Composite sample:

Minimum number of specimen for composite sample: 10 soft parts without pallial fluid.

b) Single specimen sample: soft part of one mussel without pallial fluid.

Range of shell length: 4-5 cm

Yield of soft part from one mussel: 1-2 g FW (After the spawning season the yield can be much less)

Equipment needed:

1) 2-4 plastic knives made from high density polyethylene, or similar, of high purity.
2) 5 Pyrex dishes, plastic sheets or similar.
3) 2-3 plastic tweezers (see Appendix A).
4) High density polyethylene bags or plastic containers with screw caps for sample preservation in deep freezer.
5) High density polyethylene sheets to cover the working table.
6) Smaller polyethylene sheets to be used as "weighing paper".
7) Balance with a precision of 0.1 g or better.
8) Length measuring device (page 40).
9) Plastic washing bottle with glass distilled water.
10) Plastic washing bottle with diluted H_2SO_4 (1:10).

Preoperational preparations:

1) Clean knives, dishes, tweezers, length measuring device and polyethylene sheets with detergents and/or diluted H_2SO_4, rinse with distilled water.
2) Cover the working area with precleaned plastic sheets.

Sample preparation procedure:

1) Clean hands carefully with detergents and rinse with water.
2) Select mussels 4-5 cm long which are, if possible, free from algal growth, etc.
3) Scrape off all foreign materials still attached to the outer shell surface with a plastic knife (No. 1) used only for this purpose, handling the mussels as little as possible.
4) Rinse each mussel with distilled water (or clean sea water) and let the water drain off.
5) Pull out the byssus ("by" in Fig. 1) which extrudes from between the closed shells on the concave side of the shells.
6) Weigh the whole mussel to the nearest 0.1 g, note the weight.
7) Insert a clean plastic knife (No. 2) into the opening from which the byssus extruded (Fig. 1) and cut the posterior adductor muscle by turning the knife as indicated in Figures 21 and 22, then cut in the other direction and open the mussel. Do not try to break the mussel open with the knife, if the two muscles are cut the mussel will open easily. Check if the byssus has been eliminated completely, if not, remove the remainder with clean tweezers.
8) Rinse the mussel with distilled water or clean sea water.
9) Loosen all tissue with a No. 2 knife and remove the soft tissue with a pair of plastic tweezers from the shell, without touching the outer part of the shell, and let all the water drain off (Fig. 22).
10) Place a clean piece of plastic "weighing paper" on the balance, weigh it and note the weight, then weigh the soft part of the mussel and calculate the FW.
11) Place the soft parts in a precleaned plastic bag or other plastic storage container.
12) Determine the length (see Fig. 21) of one shell by placing it with inner part facing the cm scale. Note length together with the total weight of the mussel and of its soft parts.

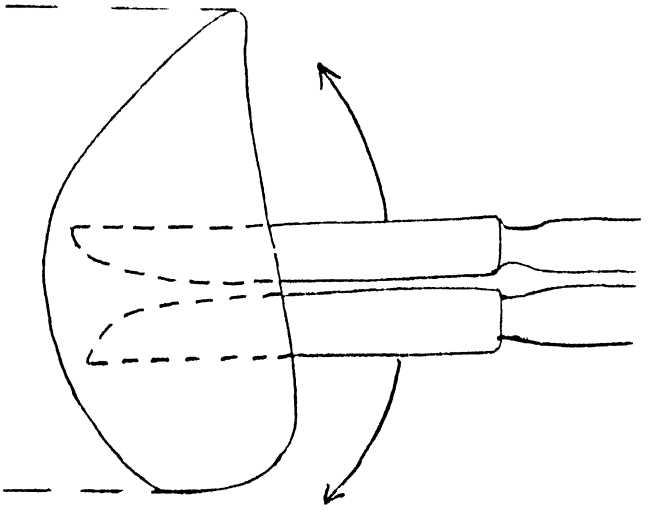


Fig. 21. Scheme which shows how to cut the two adductor muscles of Mytilus.

13a) Composite sample: if the desired sample size has been reached freeze sample as described under 5.1.2.

13b) Single specimen sample: close container and freeze as described under 5.1.2.

14) Determine DW of six soft parts prepared specially as described in section 6.2.

5.1.4 Sample preparation of Mullus

Two persons are necessary for the sample preparation. The second person may either help by taking off the fillets with tweezers, or hold the fish by the head and the tail while the first person dissects the fish. The dissection is probably best carried out on a Pyrex dish cover. In order to avoid contaminating the fillets, the skin of the fish is cut on both sides as described in step 4 before skinning the fish. Two different plastic knives and two different pairs of plastic tweezers are needed, one set for cutting and pealing off the skin, and one set of clean knives and tweezers to cut and lift the fillet from the bones. Pealing off the skin is not an easy operation, as the skin of Mullus is very delicate. The tweezers must, therefore, have a good grip to be able to get hold of the very delicate skin.

a) Composite sample:

Minimum number of specimens for composite sample: 6 fishes.

b) Single specimen sample: 1 or 2 fillets of the same fish.

Range of total length: 10–15 cm.

Yield of fillets from one fish: 8–15 g FW.

Note: fillets should be free of skin, scales and bones.

Equipment needed: Same as for Mytilus

Preoperational preparations: Same as for Mytilus

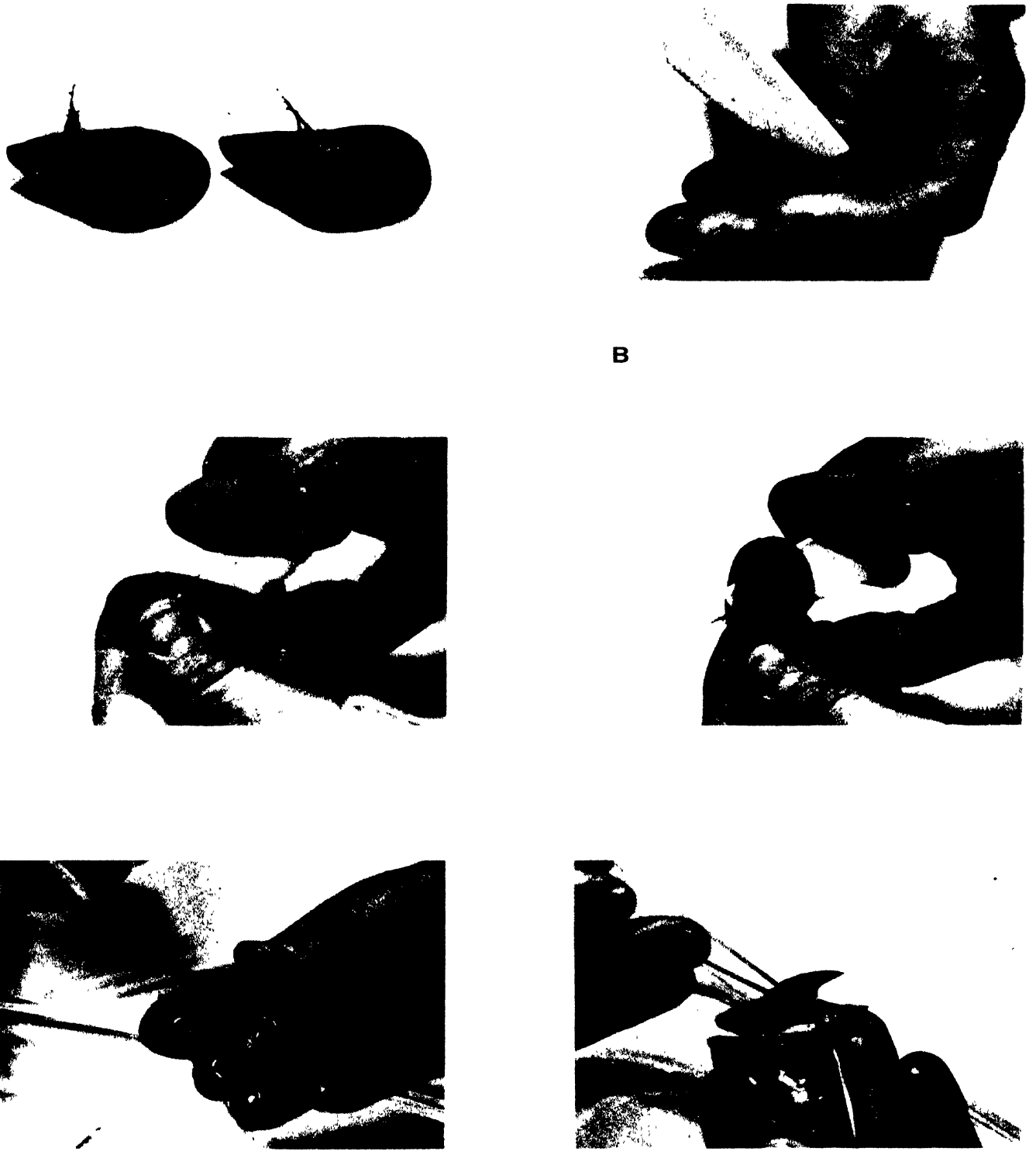


Fig. 22. Steps for opening and cleaning M. galloprovincialis in the sample preparation procedure described in the text. (A shows mussels with byssus extending upwards)

Procedure:

Note: Start with the smallest specimen (see section 7.2).

1) Determine the fork length (see Fig. 3) to the nearest mm (see page 40).

2) Weigh the fish on a clean plastic sheet to the nearest 0.1 g and calculate the fresh weight (FW).

3) Rinse the fish, first with diluted H_2SO_4 and then with distilled water.

4) Remove the pectoral fin (Fig. 23) and cut the fish's skin with knife No. 1 near the dorsal fins, starting from the head to the tail as shown in Figures 23 and 24. Then cut near the gills across the body, along the ventral edge from the gills to the tail and finally across the body near the tail. These four cuts should be carried out first on one side and then the other side of the fish taking care not to cut too deep into the fillet in order to avoid cutting into the viscera. It is advisable that a second person hold the fish by the head and tail during the operation.

5) Pull the skin from the flesh with the tweezers, taking care that the outer skin does not contaminate the flesh.

6) With clean knife No. 2, cut the fillet from the vertebral column starting from the cut near the gills while a second person is holding the head and the tail of the fish. Lift the fillet with the pair of tweezers No. 2 so that the fillet will not touch the Pyrex vessel or other parts of the fish.

7) Weigh a clean piece of plastic "weighing paper", note the weight and place the first fillet on it.

8) Put the fish, skin side upwards, on a still clean surface of the Pyrex dish or in a second clean Pyrex dish and cut the second fillet from the backbone as described in step 6.

9) Add the second fillet to the first on the balance and determine the weight of the two fillets. Note the value and calculate the FW. In composite sample it is necessary to reduce the weight of the two fillets to that of the smallest fish.

10a) Composite sample: Place the fillets into a cleaned polyethylene plastic bag or plastic container. When the total weight of the fillets of six or more fish have reached the weight necessary for the chemical analyses, press out the air from the bag, seal it with the 'sample identification note' (see page 41) and deep-freeze.

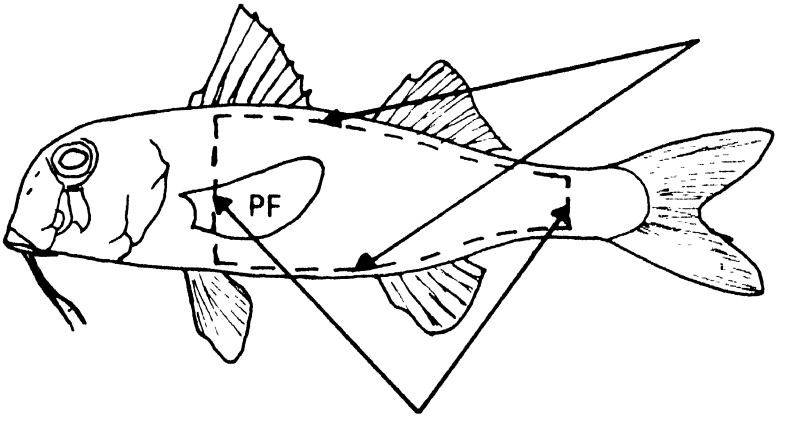


Fig. 23 Arrows show where to cut the skin on *Mullus barbatus* with knife no. in order to pull the skin off. pf = pectoral fin

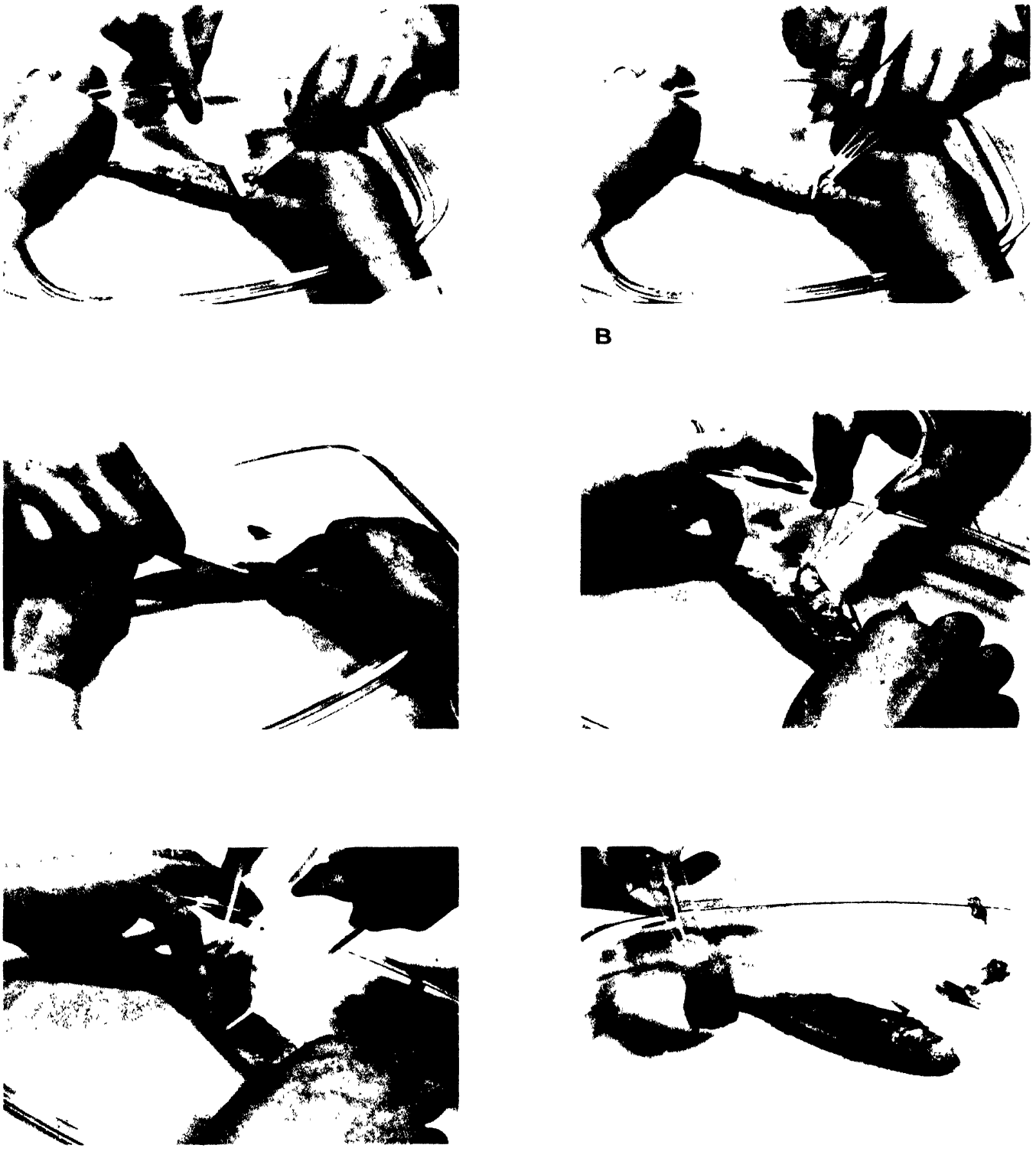


Fig. 24. Steps for removing pectoral fin (A) and skin (D) in order to obtain a fillet (E and F) in the sample preparation described in the text.

10b) Single specimen sample: Place one or both fillets in a container.

11) Prepare the data sheet as described in section 4.6.

12) Determine the dry weight as described in section 6.2.

5.1.5 Sample preparation of tuna and swordfish

The bluefin tuna and its substitute, the swordfish, will be sampled for heavy metals, especially Hg and Cd. Since both species are rather large, it will be very difficult, if not impossible, to obtain composite samples. Analyses will, therefore, be made on single specimens. In order to avoid spoiling large tuna or swordfish for subsequent sale, subsample may be taken near the tail which normally is cut off before marketing the fish. Whenever possible more than one fish should be sampled. Samples from specimens of about the same length can be pooled, if necessary. Samples from specimens of different size should never be pooled.

Sample characteristics:

Minimum number of specimen: Not stated but it is desirable to analyse at least three individual fishes. Composite samples may be prepared from tuna or swordfish which are in the same length range, i.e. ± 5 % of the total length.

Range of total length: unspecified.

Equipment needed: Same as for Mytilus

Preoperational preparation: Same as for Mutilus

Procedure:

1) Rinse the slice of tuna or swordfish with diluted H_2SO_4 and distilled water.
2) Place the slice on a clean Pyrex dish and remove the skin and bones which may be present. If the tissue sample has been cut with a metal knife during the sampling of the fish, cut thin slices from both surfaces with a plastic knife in order to obtain a clean surface not contaminated with metals.
3) Weigh a precleaned plastic bag, note the weight, place the cleaned slice in the bag, reweigh the bag with its contents, and calculate the FW.
4) Seal the samples, include the sample identification note (p.41) and deep-freeze.
5) Prepare data sheets as described in section 4.6.
6) Determine dry weight on part of the sample according to section 6.2.

5.2 Sample preparation for analyses of chlorinated hydrocarbons

5.2.1 Selection and cleaning of instruments and materials

All handling and sorting gear used during the preparation of samples for analysis of Chl-HCs must be of metal which can easily be cleaned, such as stainless steel, anodized aluminium or Pyrex glass. In order to prevent sample contamination during sampling and handling all plastics, rubber and especially paper products (tissue paper, plotting paper, filter paper, etc.) must be absolutely avoided, unless these items have been tested specifically for contamination hazards (Farrington et al., 1972). Contamination by PCBs is especiall

serious, since PCBs are very widely used in industry. Often the plastics themselves are PCB-free, but the surface has been contaminated, as PCBs are commonly used as parting agent on the surface of the moulds used for forming the plastics. Samples therefore have to be wrapped in precleaned heavy duty Al-foil. The gear, instruments and Al-foils can be cleaned with 95% ethanol of a high purity grade, or the solvents (hexane, etc.) used in the extraction of the Chl-HCs during analysis. The precleaned equipment is then oven-dried overnight. Heating instruments, etc. at 450°C for eight hours will also decontaminate efficiently since all organic substances are burned off. Sampling and subsampling has to be carried out in a clean dust-free room.

The working table may be covered with precleaned glass sheets or precleaned heavy duty Al-foil. Precleaned Pyrex dishes, or porcelain dishes and glass plates may serve as working surfaces, temporary storage vessels, etc. Precleaned and/or oven-heated (450°C, 8 hr) watch glasses can serve as "weighing papers", since normal weighing paper cannot be used due to the PCB contamination hazard. Grice et al. (1972) have listed most of the precautions necessary to avoid contamination. No gloves will be used during the sample preparation in order to avoid contamination from rubber or plastics. However, the hands have to be carefully cleaned with detergents. Further precautions are given in PAM (U.S.Dep.Health Ed., 1975) and EPA Pesticide Manual (Thompson, 1974). Distill the water over the permanganate.

5.2.2 Sample preparation for deep-freezer storage

The general procedure for deep-freezing the samples is the same for the different species and practically identical to the procedure described in section 5.1.2. except that instead of polyethylene, Al-foil, precleaned with high purity alcohol, is used to wrap the samples.

5.2.3 Sample preparation of Mytilus

The general precautions already mentioned in the section dealing with the sample preparation of Mytilus for the metal analysis are also valid here.

Sample characteristics: Same as in section 5.1.3.

Equipment needed:

1) Two stainless steel knives or similar with round tip to facilitate the loosening of the soft part of the mussel from the shells and one for cutting loose the soft parts from the shells.
2) Five Pyrex dishes.
3) 2-3 tweezers of stainless steel or other non-rusting metal.
4) Glass sheet or heavy duty Al-foil to cover working area.
5) Pieces of Al-foil for weighing the soft parts of the mussels.
6) A balance with a precision better than 0.1 g.
7) Glass wash bottle with glass distilled water.
8) Glass wash bottle with 95% alcohol.
9) A length measuring board (see page 40).
10) Two sheets of Al-foil for each sample.

Pre-operational preparations:

1) Wash hands carefully with detergents (no gloves must be used during the sample preparation).

2) Clean knives, tweezers, dishes, Al-foil etc. with 95% high purity grade ethanol, dry in oven or where possible burn off the organic material by heating to 450°C for eight hours.

3) Cover working area with precleaned Al-foil or precleaned glass sheet; or use one or more Pyrex dishes as working area.

Procedure:

1) Same as for metal analysis.

2) Same as for metal analysis.

3) Same as for metal analysis but use stainless steel knife No. 1.

4) Rinse the outside of the mussel with the ethanol from the washing bottle.

5) Same as for metal analysis.

6) Weigh the whole mussel to the nearest 0.1 g.

7) Same as for metal analysis but use stainless steel knife No. 2.

8) Rinse the inside of the mussel with the ethanol from the washing bottle, holding the mussel as shown in Fig. 21.

9) Same as for metal analysis, but use stainless steel knife and tweezers No. 2.

10) Weigh the soft part of the mussel on a precleaned piece of Al-foil and calculate the FW.

11) Same as for metals, but use precleaned Al-foil as container.

12) Same as for metals, but use precleaned Al-foil as container.

13) Same as for metals, but use precleaned Al-foil as container.

5.2.4 Sample preparation of Parapenaeus

The meat in the "tail" or abdomen (Fig. 2) of at least 10 shrimps, length 8-10 cm, will serve as a composite sample. Where the shrimp is not available it may be substituted by samples taken from the chelae of the crab Carcinus mediterraneus (see section 5.2.5).

Sample characteristics:

Minimum number of specimens for composite sample: 10 shrimps.

Range of total length: 8-10 cm.

Yield of abdomen muscle from one shrimp: 5-8 g FW.

Equipment needed: Same as for Mytilus.

Preoperational preparations: Same as for Mytilus.

Procedure: (If the shrimp sample has been partially prepared aboard, start with step 4).

Start with the smallest shrimp so that the samples can be cut all to the same weight (see section 7.2).

1) Determine the length of the shrimp, i.e. from rostrum to uropod (Fig. 2).

2) Weigh the shrimp on a piece of precleaned Al-foil and calculate the fresh weight (FW).

3) Separate the abdomen from the cephalothorax and the "tail" (telson and uropod) with a metal knife, taking care that no viscera remain in the abdomen (Fig. 2).

4) Pull out all legs (pleopods) (Fig. 2).

5) Take the abdomen with the ventral side up and cut with a metal knife along the edges of the sterinites (Fig. 25); lift the sterinites off with a pair of metal tweezers and discard.

6) Loosen with a new clean knife, the abdomen muscle and lift it from the exosceleton with a clean pair of metal tweezers.

7) Weigh the abdomen muscle on a precleaned piece of Al-foil and calculate the FW.

8a) Composite sample: Place the abdomen muscle on a precleaned piece of Al-foil, when enough pieces have been prepared, close the Al-foil, add the sample identification note (page 41) and deep-freeze.

8b) Single specimen sample: Wrap, add sample identification note (p.41) and freeze the muscle

9) Determine the dry weight as described under section 6.2.

5.2.5 Sample preparation of Carcinus

Sample characteristics:

Minimum number of crabs for composite sample: 10 crabs.

Range of carapace length and width: 2-4 cm.

Yield of tissue from chelae: 0.3-0.5 g FW.

Equipment needed: Same as for Mytilus, but instead of the knives, a pair of stainless steel scissors.

Preoperational preparations: Same as for Mytilus.

Procedure:

1) If the crabs are still intact determine the length and width of the carapace.

2) Weigh a piece of precleaned Al-foil.

3) Remove the movable finger or pincer from the chelae and scrape out the muscle tissue. Place the muscle tissue on the Al-foil on the balance.

4) Open the chelae with the scissors by cutting along one edge and remove the muscle tissue. Place the tissue on the precleaned Al-foil on the balance.

5) When enough material has been collected determine the weight of the material and proceed as described under step 10 of the procedure for Mytilus.

5.2.6 Sample preparation of Mullus

The sample preparation of Mullus barbatus proceeds in the same way as described in section 5.1.4 only that all plastics are replaced by metal (stainless steel) instruments, chemically resistant glass and Al-foil. The operators should work with bare hands cleaned with detergents.

Sample characteristics: Same as for metals (section 5.1.4).

Equipment needed: Same as for Mytilus (section 5.2.3).

Preoperational preparations: Same as for Mytilus.

Procedure:

Start with the smallest fish so the filets for the composite sample can be cut to the same weight, if necessary (see section 7.2).

1) Same as for the metal analysis.
2) Same as for the metal analysis.
3) Rinse the fish with 95% alcohol.
4) Same as for the metal analysis, but use metal knives and tweezers instead of plastic instruments.
5) Same as for the metal analysis, but use metal knives and tweezers instead of plastic instruments.
6) Same as for the metal analysis, but use metal knives and tweezers instead of plastic instruments.
7) Same as for metal analysis, but use precleaned Al-foil as "weighing paper".
8) Same as for metal analysis, but use precleaned Al-foil as "weighing paper".
9) Same as for metal analysis, but use precleaned Al-foil as "weighing paper".
10) Prepare samples for deep-freezer storage as described under 5.2.2.
11) Same as for metal analysis.
12) Same as for metal analysis.

5.3 Sample preparation for analyses of metals and Chl-HCs of other marine organisms

Molluscs, crustaceans and fish can be prepared according to the precedure described above for the respective species. Plankton samples can be prepared following indications by Martin and Knauer (1973), Harvey et al. (1974) and Grice et al. (1972).

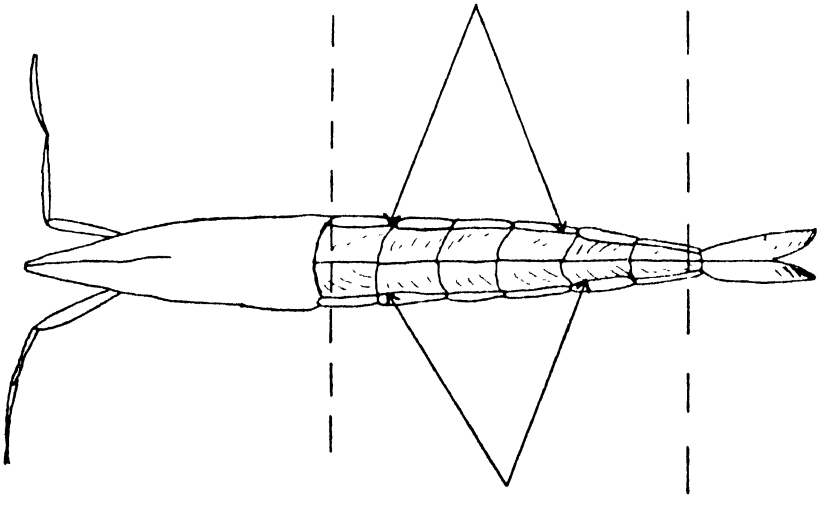


Fig. 25 Arrows indicate where to cut shrimps after the legs have been removed, in order to remove the tail muscle.

6. ANALYTICAL PROCEDURES

The expert consultation recommended atomic absorption spectrophotometry (AAS) as basic analytical instruments for the analysis of metals and inorganic constituents and gas chromatography (GC) for the monitoring of chlorinated hydrocarbons (Chl-HCs).

Neutron activation analysis (NAA), X-ray fluorescence (XRF) etc. are recognized as alternative methods. Their use especially with regard to multi-element analysis should however be encouraged, since these techniques will also supply the necessary independent methods for estimating the accuracy of the analytical results obtained with the AAS.

It was further recommended that the use of existing or the creation of new gas chromatography-mass spectroscopy units should be taken into consideration in order to ascertain and facilitate the identification of the substances revealed with the GC.

Both for the analyses of metals and for Chl-HCs no standard analytical techniques will be used in these pilot projects. However, this outline will make some suggestions for methods which may be especially useful to those who have not yet set up a method of their own.

Several of the chemical determinations require a pretreatment of the sample. For metals this pretreatment consists in destruction of the organic matter of the sample and the transformation of organically bound metals into inorganic ions or free metals. In the case of Chl-HC analysis, separation steps have to precede the actual determinations.

The greatest problem in the chemical analysis of marine organisms is the lack of uniformity from species to species and hence the "matrix", i.e. the chemical composition of the samples, may be different in each species investigated (Windom,1972). An estimation of the accuracy of the results obtained by an analytical method is,therefore,of greatest importance. The accuracy of analytical results can be checked in two ways:

1) by analysing a standard reference material of a well documented chemical composition which is chemically similar (same matrix) to the samples under investigation.

2) by analysing a series of identical samples with independent methodologies.

Another important aspect of monitoring is (1) the estimation of the analytical variation (precision) i.e. the variance or random error of the entire analytical determination starting from the pretreatment to actual determination and (2) the estimation of the biological variation of the pollutant's concentration which is caused by the variation of intrinsic biological or environmental factors. It should be realized that without a good estimation of these sources of variation it will be impossible to establish statistically significant differences between samples taken from different locations or from the same location at different times, i.e. trends in pollution in time and space. It is obvious that the determination carried out on a pooled sample will supply only a value which is equivalent to a mean and does not give any indication about the variation between specimens and hence it is not sufficient to draw conclusions about the differences between samples. The analyses of pooled samples should therefore, whenever possible be supplemented with a certain number of determinations designed to estimate the different sources of variation (see section 7).

6.1 Contamination of glassware and its avoidance

The changes of the sample due to contamination and loss present a major problem in trace element analysis. Zinc, lead and copper are present everywhere and many laboratories are

contaminated by Hg. Bothner and Robertson (1975) showed that Hg can penetrate from Hg contaminated laboratory floor into polyethylene bottles contaminating the sea water they contain. Although biological materials usually have Hg concentrations 100 to 1000 times higher than sea water possible Hg contamination hazards should be contemplated and, if necessary eliminated. Slavin et al. (1975) analysing for nine heavy metals encountered severe contamination problems which could be solved after washing all glassware with nitric acid. In order to minimize contamination from the glassware Julshamn and Braekkan (1975) washed all glassware with a mixture of 30 percent concentrated HCl and 10 percent H_2O_2 in distilled water. They then rinsed with diluted HCl and deionized water, and finally dried at 90°C. However, often such elaborated cleaning procedures are not necessary and it is sufficient to clean the glassware by soaking it overnight in a good detergent and rinse several times with distilled water. The traditional chromic-sulfuric acid mixture must be avoided at all costs, as it will contaminate the glass surface with Cr which is very difficult to remove (Thiers, 1957).

Sperling (1975) found that he had to clean his pipette tips prior to use with acid to leach out Cd. He also observed that different brands of polypropylene tubes used for the digestion of the biological material gave different Cd blank values equivalent to 0.1 to 0.2 µg Cd/l. Sommerfeld et al. (1975) suggest rinsing pipettes with 1% ultrapure HCl three times in order to reduce Zn and Fe contamination. Robertson (1968), Bernhard and Piro (1971) and Everson (1972) showed that rubber and certain PVCs can contaminate solutions passing through them. They suggest that only polyethylene, Teflon, Tygon or tubing which has been proven not to leach heavy metals should be used. For a recent review consult Robertson (1972).

6.2 Determination of dry weight in biological material

The concentration of elements and substances are usually refered to fresh weight (FW) and dry weight (DW), and both should, if possible, be determined. FW or live weight is not easily defined, since marine organisms lose water more or less rapidly after being taken from the sea water. On the other hand most biological models refer to FW because it is easier to compare with volume measurements. DW determinations also present problems, because the requirement that the tissues should be dried until constant weight is reached, can not always be satisfied, especially when large amounts of lipids are present as is often the case in fish tissue. Reporting data on ash weight only has slight meaning.

The determination of the DW requires that the sample be dried at 110°C until constant weight is obtained (usually after 24 to 48 hours). Repeated weight determinations of the sample are necessary to establish whether a constant weight has been reached.

Equipment needed:

1) Weighing glass with a ground stopper
2) Desiccator
3) Analytical balance
4) Drying oven with thermometer

Procedure:

1) A clean weighing glass, with the ground stopper removed, is placed into the drying oven (110°C, 2 hours), using a pair of clean pincers. It is important to use the pincers every time the glass is touched, to avoid fingerprints and dirt particles on the weighing glass.

2) The stopper and the glass is put into the desiccator to cool.

3) The empty glass and stopper are then carefully weighed on the analytical balance. The obtained weight is the weight of the dried, empty weighing glass and stopper (W 1). Note the weight.

4) Remove the stopper and place about 1-2 g material into the weighing glass and replace the stopper.

5) Determine carefully the weight of the weighing glass plus stopper. The obtained weight is the FW of the tissue plus the weight of the glass and stopper (W 2).

6) Place the glass in the drying oven (110°C) removing the stopper and placing it also in the drying oven.

7) After 24 hours replace the stopper on the glass, remove the glass with stopper from the drying oven and place it into a desiccator to cool.

8) Weigh the glass (W 3), note the weight.

9) Return the glass into the drying oven (110°C), remove the stopper.

10) After 24 hours repeat step 7 and 8. If the weight W 3 does not change the determination is terminated.

$$W_2 - W_1 = FW \quad ; \quad W_3 - W_1 = DW$$

6.3 Destruction of organic matter in biological samples

AAS requires that the organic matter of the samples is destroyed. This can be achieved by dry or wet ashing (combustion). In general wet combustion is prefered since heating the materials to more than 450°C in order to combust the organic matter will cause losses of the more volatile compounds. While there seems to be no doubt that volatile organic metal compounds can be lost during drying and dry-ashing, the actual experimental data published are contradictory. Strohal et al. (1969) and Doshi et al. (1969) investigated the loss of several elements by allowing marine organisms to accumulate radio-isotopes from an artificial environment or from isotopes injected into the organisms. Strohal et al. found that even at low temperatures (110°C) losses ranged from 9 (Zn) to 14% (Mn and Co). At 450°C the range extends from 23% (Zn) to 15% (Mn). Doshi et al. observed similar but not so high losses at 400°C ranging from 9% (Zn and Co) to only 4% (Mn). On the other hand more recently Van Raaphorst et al. (1974) investigated loss of Zn and Co from isotope marked mussels and seaweeds. They observed no zinc or cobalt losses due to volatilization even at temperatures as high as 1000°C. Contrary to other authors who observed considerable adsorption of elements to the porcelain crucibles used, Van Raaphorst et al. noted only slight adsorption even at high temperatures.

The results of Van Raaphorst et al. are not easy to interprete, especially if one takes into consideration relative low melting and boiling points of certain inorganic substances, not to speak of the low melting and boiling points of organic metal compounds. Experiments with graphite furnace used in flameless AAS show quite clearly that losses of elements occur. In fact, the determination of many elements is based on the quantitative volatilization in the furnace. Sperling (1975), for example, showed that $CdCl_2$ is lost rapidly after heating for about one minute at temperatures higher than 420°C. The presence of HNO_3/H_2SO_4 or $HNO_3/HClO_4$ mixtures, as used in wet digestion, retards the loss slightly.

Dry ashing at low temperature with excited oxygen is not recommended, since losses of volatile Hg compounds have been observed by Pillay et al. (1971). Parslow (1973) observed losses (15%) of Hg when drying bird livers at 105 . These conflicting result seems to indicate that during the heating of the samples the various components of matrix react with each other changing thus the volatility. In fact Ediger (1975) in an attempt to decrease the volatility of analyses in order to prevent their volatilization during the charring step and to increase the volatility of organic matrixes thus promoting their removal before atomization in the graphite furnace observed, for example, that the addition of small amount of Ni or Cu decreases the selenium volatilization in NBS Bovine Liver Standards. In model experiments practically no losses of Se occurred when 20 µg Ni, an amount which in certain analytical procedures could easily result from contamination, was added to a sample containing 2 ng of Se in deionized water and the sample was heated up to 1200°C. Decreases in volatility were also observed for As, Ge, Ga, Cd and Hg under various experimental conditio Since in complicated matrixes these 'matrix transformations' apparently can not easily be foreseen recovery experiments with the different matrixes encountered, should be undertaken to ascertain that quantitative recovery is guaranteed under the conditions used in the digestion procedures.

In order to guarantee a uniform sample treatment by all participants it would be desirable that the same wet combustion treatments be used for all elements. It is, therefore, recommended that in this pilot project only closed digestion vessels be used and that before starting the analysis of the samples a recovery investigation should be carried out in order to verify that the experimental conditions are satisfactory. It should also be remembered that blank digestions must run with each set of analyses to compensate for contamination hazards from the chemicals used in the digestion process.

A few general recommendations to be followed during wet combustion may be listed here (Windom, 1972): When using fuming nitric acid care must be exercised to avoid ignition upon heating. After adding the nitric acid allow the sample to stand for 30 minutes without heating. If the sample should ignite, extinguish it immediately with double distilled water to avoid volatilization and hence loss of the analytes. If the sample can not be extinguished immediately it must be discarded and a new smaller one mineralized.

The destruction of organic matter has been reviewed recently by Gorsuch (1970), Christian (1969), Reimers et al. (1973) and especially with regard to Hg by Ure (1975).

6.3.1 Explosion hazard

If too high amounts of organic materials are placed in close vessels, e.g. instead of the amount of material in FW the same amount in dry weight (DW) is used. The vessels, especially the plastic bottles used in the Adrian's procedure (see below) may burst. Therefore, all digestion procedures must be carried out with the appropriate precautions necessary when working with hot acids. For example fume hoods must be closed when the vessels are heated.

Defect plastic bottles must be discarded and bottles which have been used a certain amount of time must be replaced before they burst (Aitsetmüller et al., 1973).

6.3.2 Liquid pressure decomposition of organic matter for metals

For the destruction of the organic matter strong oxidizing agents are used. Nitric and sulphuric acids, sometimes followed by oxidation with potassium permananate, hydrogen peroxide and/or pottassium persulphate have been employed (Bouchard, 1973; Barber et al., 1972; Cutshall and Naidu, 1972; Stainton, 1971; and Uthe et al., 1974). Since many organic Hg-compounds are highly volatile, open vessels such as Kjeldahl flasks can not be used. Not even reflux systems can prevent loss of volatile metal compounds, only closed systems are reliable (Holak et al., 1972; Stoeppler and Backhaus, i.pr.). Several firms manufacture containers, with Teflon lining or fitted with Teflon crucibles with airtight covers, which are easy to operate. Digestion temperatures higher than 100°C facilitates the dissolution of the organic matter and shortens the reaction time, and hence the decomposition procedure. Several crucibles can be processed together, increasing the efficiency of the wet combustion work schedule. As in other closed system equipment the only disadvantage consists in their possibility to explode or to be damaged when too large amounts of organic material are processed. The digestion has, therefore, always to proceed in a fume hood with closed hood window and adequate protection, in order to avoid damage caused by spills of the highly corrosive acids. Samples should never be kept in the containers for any length of time after digestion, since the strong digestion solution will attack the container material, and together with the impurities it contains will dissolve in the digestion solution presenting a contamination hazard. Therefore, only as many digestions as can immediately be analysed should be set up at a time.

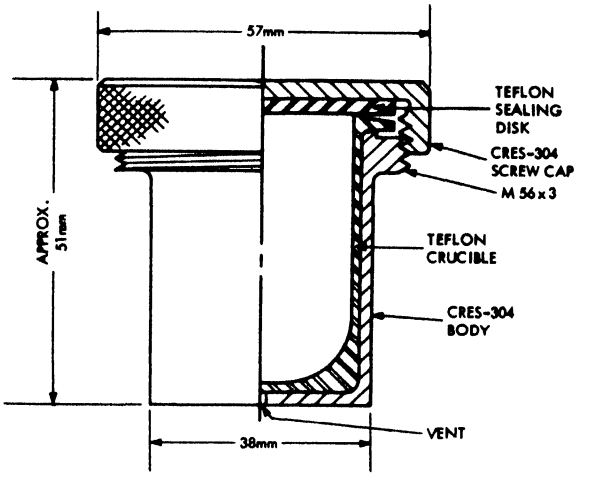


Fig. 26 Decomposition vessel (Bernas, 1968)
(Reproduced with kind permission of the author).

The closed vessels are nowadays easily available and in this outline it is recommended to use them exclusively.

It is also important not to exceed the quantities of organic materials recommended below. Since excess nitric acid has to be eliminated in order to avoid interference in the determinations, it is suggested that in preliminary experiments the amount of acid necessary to destroy the organic matrix is determined for the few matrixes used in this project.

Cleaning of the crucible before and between digestions.

Clean with detergents, if necessary, and rinse with distilled water, then run a digestion procedure without adding the sample. Analyse the solution for the analyte according to the analytical procedure selected. If the blank value is high repeat the 'blank digestion'.

6.3.2.1 Wet combustion in closed Teflon crucibles

Holak et al. (1972) have described the use of a closed Teflon crucible in a steel block for the digestion of fish tissues. Their procedure is the following (slightly modified):

Apparatus

Digestion vessel - (Uni-Seal Decomposition Vessels, P.O. Box 9463, Haifa, Israel. Fig.26)

Oven or muffle furnace

Reagents

HNO_3, high purity electronic grade, 65%

Predigestion experiments

Determine the minimum amount of nitric acid necessary to destroy completely the organic matter for every new matrix by adding to a 1 g FW sample increasing amounts of acid from 2 ml to not more than 6 ml HNO_3.

Procedure

Weigh ca 1 g FW sample into a Teflon digestion vessel. Add the amount of HNO_3 sufficient for the digestion of the organic matrix and close the vessel, tightening the lid containing a Teflon sealing disk. Place the vessel, without tilting, into the preheated 150°C oven or muffle furnace for 30–60 minutes or until the solution is clear. (Optimum time may vary with the type of fish. If the solution is colloidal, heat until clear). Remove the vessel from the oven and let cool to room temperature. Unscrew the cap, snap on spout, and transfer the sample with aid of some distilled water into a 10 ml volumetric flask and bring up to volume with additional distilled water. Analyse within six hours.

A similar digestion apparatus is developed by Stoeppler and Backhaus (i.pr). Here several Teflon vessels complete with Teflon cover are placed in a stainless steel block. The Teflon cover is held in place by a series of steel springs. If the pressure during the combustions exceeds a certain value the Teflon cover will lift and the pressure is released without a major explosion.

Apparatus

Stainless steel blocks with Teflon crucibles with a volume of about 50 ml. (Firma H.J. Groteklaes Maschinen-und Stahlbau, 517 Jülich, Margaretenstr. 7, Federal Republic of Germany. Fig. 27).

Hot plate with thermostatic control.

Reagents

HNO_3, high purity electronic grade, 65%

Predigestion experiment

Determine as in the previous method the minimum amount of HNO_3 necessary for the distruction of the organic matrix.

Procedure

1. Place a sample of about 1 g FW into each of the Teflon crucibles.
2. Add the predetermined amount of concentrated HNO_3, cover the crucible and mount the steel cover with the springs. Tighten the screws crosswise.
3. Allow the sample to predigest for 30 minutes or longer.
4. Heat the steel block containing the Teflon crucibles to 100°C on a thermostatic controlled hot plate for 60 minutes.
5. Let the block cool by itself, or by applying cold air or placing it into a water bath; open when cool. Solution is now ready for analysis.
6. If the solution is not clear reheat for another 60 minutes at 100°C

6.3.2.2 Wet combustion in closed plastic bottles

Adrian (1971) has proposed another digestion procedure with closed vessels which allows the treatment of a large number of samples simultaneously utilizing only inexpensive equipment. Luyten et al. (1973) compared this method with other digestion procedures, showing that Adrian's procedure compares favourable with the other techniques in the determination of Cu and Zn, except when tissues with high lipid content are processed. However, since Bothner and Robertson (1975) have shown that Hg can penetrate plastics, Hg contamination hazards have to be taken into consideration.

Apparatus

Nalgene bottles or similar plastic bottles (50 to 100 ml) with screw caps,

Water bath (70°C) large enough to hold the number of bottles (10-100) to be processed

Reagents

$HClO_4$, high purity electronic grade

HNO_3, high purity electronic grade

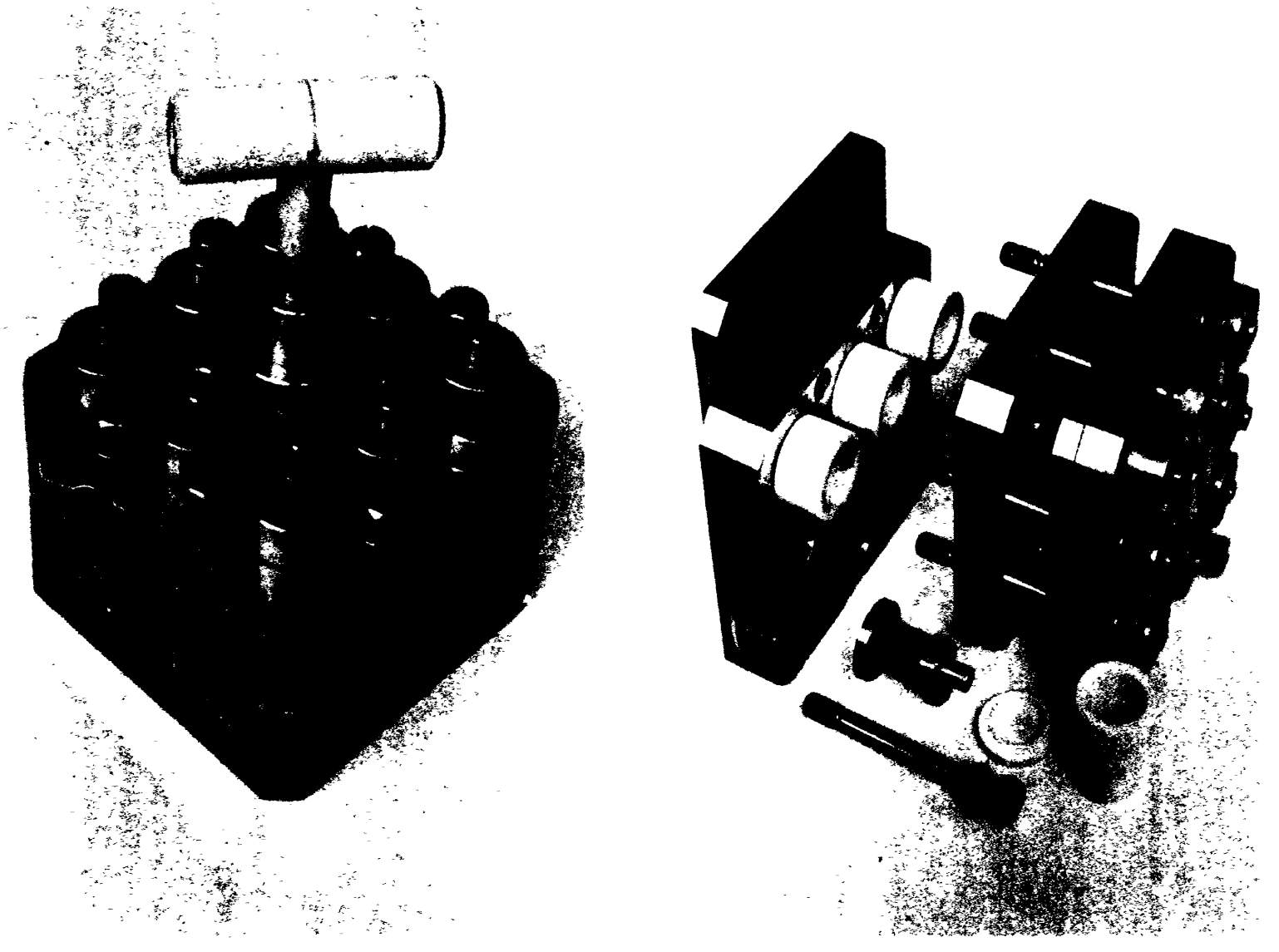


Fig. 27 Multiple vessel decomposition block reproduced with kind permission of Groteklaes Ma hinen-und Stahlbau Jülich. Dimensions of the samples steel block: 150 x 150 mm; high 117 mm

Procedure

1. Place about 5 g FW samples into each bottle
2. Add 1.5 ml $HClO_4$ and 3.5 ml HNO_3 (determine the minimum amount necessary), seal tightly and allow to predigest overnight under the fume hood
3. Place the bottles in a hot water bath (60-70°C) for 2 to 3 hours (until the solution is clear)
4. Remove the caps and add 2 to 3 ml distilled water
5. Reheat the open bottles under the fume hood to remove the excess acid which interferes in the AAS determinations.

After cooling the samples are ready for analysis. The bottles may be used as reaction vessels in the flameless aeration-reduction procedure for the determination of Hg, As and Se.

Caution: Since the plastic bottles may burst if too high pressure is developed the digestion procedure must be carried out in a closed fume hood.

6.4 Atomic Absorption Spectrophotometry

The application of atomic absorption spectrophotometry (AAS) for the determination of elements in biological materials consists in introducing, usually after wet combustion, a digest of the material through a burner of an atomic absorption spectrophotometer where the sample is atomized by a flame (Flame AAS). A light beam emitted by a hollow cathode lamp with a cathode made from the element to be analysed is passed through the flame. When a sample is evaporised in the flame, its atoms absorb the lines emitted by the cathode and thus reduces the signal of the spectral line projected with the aid of a monochromator into a phototube. The reduction of the signal is proportional to the amount of the element in the sample. However, non-atomic absorption of the line emitted by the hollow cathode lamp can also occur, especially when samples containing high concentrations of other substances are burned in a low temperature flame. This non-atomic absorption is caused by two processes: molecular absorption and light scattering.

Molecular absorption occurs when the flame temperature is not high enough to transform all molecules into atoms. Higher flame temperature causes more molecules to disintegrate but they also produce a higher number of ions thus reducing the atomic absorption and hence the sensitivity.

Light scattering is caused by particles present in the sample solution. These particles are produced in the flame when the organic material is not completely destroyed in the previous combustion or by the high solid content in the aspirated sample. They reduce the signal which arrives at the photomultiplier. Both types of non-atomic absorption occur over a wide range of wavelength and are greatly increased at shorter wavelength.

Three methods may be employed to reduce or compensate for the non-atomic absorption. One is a chemical separation before introducing the sample solution into the flame. Another method consists in applying a correction (background correction) to the atomic absorption measurement and the third requires that the standards are prepared in solutions which approximate the matrix concentrations. The absorption measurement of the line source (e.g. the hollow cathode lamp) is the sum of the atomic absorption and the non-atomic absorption. The background correction of the absorption due to light scattering and molecular absorption can be achieved by determining the absorption at the selected wavelength of a continuous spectrum emitting light source (hydrogen filled hollow cathode lamp or deuterium arc lamp). In practice this means that both (i) the absorption of the selected line emitted from the cathode lamp and (ii) the absorption of the continuous spectrum is determined.

In modern double beam instruments these two measurements are carried out simultaneously so that in very short succession (20 to 150 times per second) either light of the cathode lamp or the continuous source pass through the flame. The data processing unit which receives the output from the photomultiplier is synchronized with the modulated light sources so that the signal from the cathode lamp can be distinguished from the signal of the continuous source making an automatic correction for non-atomic absorption possible (automatic background correction).

The third methodology used in correcting for non-atomic absorption consists in preparing the standards in solutions which closely resemble the chemical composition of the sample under investigation or using the standard addition method for calibration. This requires that the concentration of potential interfering elements is known (e.g. Windom, 1972), or that known amounts of the element to be analysed are (after pretreatment) added to subsamples of the original sample. From the data obtained, a calibration curve is constructed which, when extrapolated to zero absorption, allows the estimation of the concentration of the element in the sample.

Preparing a solution which simulates the often complicated matrix of environmental samples is not easy, since viscosity, surface tension and complex chemical composition can often not be matched accurately enough. It also requires additional chemical analyses, expecially of alkali and alkaline earth metals which are present in high concentrations in marine samples. Also the chemicals used to simulate the matrix constitute contamination hazards. An indication of the approximate concentrations of Na, K, Ca, Mg, Cl, S, P and Fe in marine organisms can be obtained from Table III.

Besides the normal flame AAS two other AAS techniques are of interest. Elements and compounds which are volatile at room temperature such as metallic Hg, and the hydrides of As, Se, Pb, Sb, etc. can be introduced, after the necessary transformation to volatile state, into the light beam. The absorption of metallic Hg is then determined without a flame while the hydrides are atomized by a flame. This chemical transformation, which eliminates many matrix interferences, is used mainly for the determination of Hg, As and Se.

Another flameless AAS technique relies on the volatilization of elements at high temperature. This is achieved by heating a small sample (5–100 µl) in a graphite furnace in the absorption light pass. Comparing the sensitivities of the different methods show that the determination with the flameless AAS have lower detection limits (DL) except when matrix interferences in the graphite furnace occur. Another advantage of the graphite furnace is the small sample size. In Table IV an example is given of the DLs of the different AAS methods together with the concentration range which are typical for marine organisms. In many cases the sensitivity of the flame AAS should be sufficient for the determinations.

In conclusion it can be said that despite the fact that many authors have without apparent difficulties analysed many different elements with flameless and flame AAS, often a wide spread of data is observed for the same elements in intercalibration exercises (see e.g. ICES, 1974). The determination of trace elements is not necessarily an easy undertaking and many factors can influence the quality of the results. A good reproducibility or precision of the results obtained in one laboratory is an indication that the many factors which may influence the outcome of the determination are under control, but still systematic errors may be a problem. These systematic errors can only be determined by intercalibration, reference to a certified standard, or analysis with different independent methodologies.

Table III

Major constituents (Na, K, Ca, Mg, Cl, S, P) in some marine organisms in µg element/g FW

	Na	K	Ca	Mg	Cl	S	P	Ref.
sea water 36‰ S	10600	380	400	1300	18000	800	10^{-5}	(6)
Ulva lactuca*	3100–5600	4100–4700	3500–17200	4100–4500	200	800–34300	–	(1)
Calanus finmarchicus	5400–6500	2900	360– 400	240– 300	10500–11800	1400– 1500	1300–1500	(2)
Plankton, 76 µ-net*	11500	7900	1200	1300	–	–	–	(8)
Plankton, 360 µ-net*	8000	1000	2000	900	–	–	–	(8)
Euphausids *	5600	1200	900	800	–	–	–	(8)
Copepods *	7400	1200	800	850	–	–	–	(8)
Radiolarians *	10300	1100	850	1100	–	–	–	(8)
Penaeus sp. soft pt.	1000–1100	3800–4100	500–1400	400– 500	–	–	1500–2200	(3)
Mytilus edulis, muscle	1400	9000	130	700	1100	100	3600	(4)
Cardium edulis, muscle	5100	3050	410	930	–	–	–	(4)
Patella vulgaris, podium	4200	2400	1000	760	–	–	–	(4)
Sepia officinalis, muscle	600	5260	60	310	650	50	2190	(4)
Ostrea edulis	6500	2580	1850	410	–	–	–	(4)
Engraulis encrasicolus	1300	2700	3000	540	2100	2600	2200	(5)
Sardina pilchardus	930	5000	2300	460	1700	2700	2200	(5)
Merluccius vulgaris	750	5000	750	4500	2200	2100	1000	(5)
Mugil cephalus	1000	3200	300	300	–	–	1700	(5)
Scomber australasicus	1000	4200	200	400	–	–	2700	(5)
Fundulus heteroclitus *	1160–2100	3320–3930	11900–25000	530–800	–	–	–	(7)

* estimates from DW or AW

Ref: Vinogradov (1953) (1) page 65, (2) p. 387, (3) p. 383, (4) p. 291–292, (5) p. 506–509, (6) Sverdrup et al. (1942) (7) Eisler and La Roche (1972), (8) Martin and Knauer (1973)

Table IV

Typical concentration (ng/g FW) in marine organisms
and detection limits (DL) in flame and flameless AAS (ng/ml)

Element	conc. in organisms	flame relative	graphite furnace relative	graphite furnace absolute	aerat.-reduction relative
Hg	100– 3000				0.05–0.1
Cd	10– 1500	2	0.003–0.02	0.0003–0.0005	
Zn	3000–100000	1 – 3	0.001–0.02	0.0001–0.00008	
Pb	50– 5000	10 – 30	0.05 –1	0.005	
Cu	200–50000	1 – 8	0.05 –1	0.005 –0.007	
Cr	200–10000	3 – 8	0.1 –1	0.01 –0.005	
Mn	100–20000	2 – 8	0.01–0.1	0.001 –0.0005	
As	200–10000				0.15–0.2
Se	500–2000				0.15–0.3
Na		< 0.2–1			
K		< 2 – 4			
Ca		< 2 – 2			
Mg		< 2 – 4			

6.4.1 Determination of Hg

Mercury occurs in the environment both in inorganic and organic forms. Since the organic forms of mercury are more toxic and in many organisms 60 to 95% of the mercury is organically bound (Knauer and Martin, 1972, Cumont et al., 1972) information on both the inorganic and organic mercury concentrations are needed. The determination of the organic mercurials can be carried out with gas chromatography or with AAS. Using AAS the organic mercury concentrations are obtained from the difference between the total and the inorganic mercury. Inorganic mercury is released in the presence of organic mercurials by addition of tin(II) chloride alone (Magos, 1971). On the other hand if the tin chloride is added together with a cadmium or copper salt, inorganic plus methyl-Hg and other organomercurials are released. One has, therefore, different possibilities to determine inorganic and organic Hg separately: Either by determining inorganic mercury and subtracting this value from a total Hg determination after wet digestion or from the differential release of first inorganic and then organic Hg.

6.4.1.1 Determination of total Hg

Due to the recent development of the flameless AAS methods, especially the reduction-aeration techniques, auxilary equipment kits are now commercially available (Stux and Rothery, 1971; Klemm and Fetter, 1972; Wolber and Bosshart, 1972; Kahn, 1971; Anon., 1972; etc.). These kits have greatly facilitated the Hg analysis, but, although the reduction-aeration technique is in principle simple and sensitive, in practice an accurate determination of Hg in natural samples is difficult (Ure, 1975). This is partly due to volatility of the organic mercury compounds, to contamination of the samples from air, storage and sampling equipment and to several parameters which influence the actual analytic methods.

The determination of the total mercury (Hg_T) in biological samples with AAS requires:

(1) Transformation of all organic mercurials into inorganic Hg, either by wet combustion or through the addition of Cd or Cu salt during the reduction process with $SnCl_2$.

(2) The reduction of the mercuric ion to metallic Hg with an excess of $SnCl_2$ or $SnSO_4$

(3) The volatilisation of metallic Hg at room temperature by aeration and its measurement by flameless AAS.

Step (2) and (3) describe the so-called reduction-aeration technique.

Several publications deal with the various versions of this method.

(a) Aeration with recirculation. Here the air used for the volatilisation is passed several times through the reaction vessel containing the sample in a closed system. This system consists of the reaction vessel, the AAS absorption cell, a pump and often a drier to avoid condensation of water in absorption cell (e.g. Uthe et al., 1970). This configuration has the advantages that Hg can be liberated from large volume samples, and that the

recorded absorbance will reach a plateau after some time, but often memory effects are caused by the many components of the system.

(b) Aeration without recycling. (Armstrong and Uthe, 1973, Iskander et al., 1972), In this configuration the air-Hg mixture, after passing through the AAS absorption cell, is discharged from the system. It has the advantage of a simpler system with less components in contact with air-Hg mixture, but the partition between Hg in solution and in air is not optimal. However, the memory effect is neglectable and the system can easily be rendered automatic (Armstrong and Uthe, 1973).

(c) With and without air drying. In order to avoid water condensation in the AAS absorption tube the air-Hg mixture is often dried with magnesium perchlorate, anhydrous calcium sulphate or silica gel (Hatch and Ott, 1968, Ure and Shand, 1974). The disadvantages of the driers are memory effect, loss of Hg and contamination hazards due to the chemicals used for drying. Gilbert and Hume (1973) have used heated absorption cells to prevent water condensation. Stux and Rothery (1971) let 15 to 20% of the air used for aeration bypass the reaction vessel and go directly to the absorption vessel. In this way the dry air will prevent the condensation of the absorption cell.

(d) Agitation and stirring the sample before aeration or instead of aeration. Ure and Shand (1974) agitated the sample with a fixed volume of air instead of aeration with bubbles and then passed the air-Hg mixture into the measuring cell. Under this condition no driers are needed. Stux and Rothery (1971) combined stirring with bubbling. After addition of the $SnCl_2$ or $SnSO_4$ the sample solution was stirred for 90 seconds before bubbling. The peak height becomes approximately five times greater than when the bubbling is started immediately after the addition of the Sn-salts.

Armstrong and Uthe (1973) proposed a semi-automatical Hg-determination in which the samples are first digested manually with a mixture of nitric and sulphuric acid, oxidized with $KMnO_4$ and cleared with H_2O_2. A series of treated samples are then automatically analysed with the aid of Technicon Auto-Analyser Equipment which carries out the reduction of Hg with hydroxylamine and $SnSO_4$ and equilibrate the Hg vapor with air before passing it through AAS. Also Bailay and Lo (1971), Lindstedt and Skare (1971) and others have published automated Hg analyses. The digestion is, however, always carried out manually. Automated analyses should be preferred to manual ones since they increase reproducibility. Armstrong and Uthe (1973) report a relative standard deviation of 3-8% in a concentration range of 0.100-9.000 ppm = 100-9000 µg/kg FW.

Several publications deal with possible interferences (see Ure, 1975 for a review). During the digestion of tissues from marine organisms bromides and iodines can be formed which produce interferences in the flameless Hg determination (Omang, 1973). The interference of the anions of the commonly used acids in the digestion process is neglectable, if the digested solutions are diluted. Chloride causes depression of the signal when above 4 M. In most digested samples the interferences are not serious provided that the standard solutions are made up in similar matrix. The concentration procedures for Hg from solutions reviewed by Ure (1975) may be used to collect the products of a preliminary reduction-aeration, if doubts about matrix interferences may arise. Recent reviews of Koch and Manning (1973), Westöö (1974) and Ure (1975) can be consulted for additional information.

In the following the procedures of two versions of the reduction-aeration method are described in detail. An open system using the commercial kit supplied by Varian (Stux and Rothery, 1971) following a digestion in a closed Teflon crucible or plastic bottle as described under 6.3 and a closed system following the same digestion method as published by Munns

and Holland (1971) and recommended by the 'FAO/WHO expert consultation to identify the food contaminants to be monitored and to recommend sampling plans and methodology' (FAO/WHO, 1975). The closed system version has also been adopted as official first action by the American Association of Official Analytical Chemists for the determination of Hg_T in sea food (Krinitz and Holak, 1974).

Before describing the Hg methods in detail a few general precautions and suggestions are to be mentioned. The air used in the aeration-reduction method to transport the volatile Hg into the absorption cell must be cleaned by passing it through two washing bottles filled with an acidic potassium permanganate solution prepared by mixing equal volumes of a 2% K-permanganate solution and 50% (v/v) H_2SO_4 (Topping and Pirie, 1972).

When K-permanganate has not been used in the digestion the digested sample can be reduced directly with $SnSO_4$ to elementary Hg. Unstoppered bottles containing acidic K- permanganate solution used for preparing digestion mixtures collect Hg from the contaminated ambient air (Ure, 1975).

6.4.1.1.1 Flameless Hg-determination in a closed system (Munns and Holland, 1971)

Apparatus:

a) AA-spectrophotometer with Hg lamp and gas flow-through cell complete with quartz windows, continuous spectrum lamp and pen recorder.

b) Diaphragma pump internally coated with acrylic-type plastic spray, or similar.

c) 16 gage Teflon tubing for connections.

d) Gas inlet adapter $\mathfrak{F}$ 24/40 (Kontes Glass Co. K-18100 or similar).

e) Boiling flasks 250 ml flat bottom boiling flasks with $\mathfrak{F}$ 24/40 joint, or similar

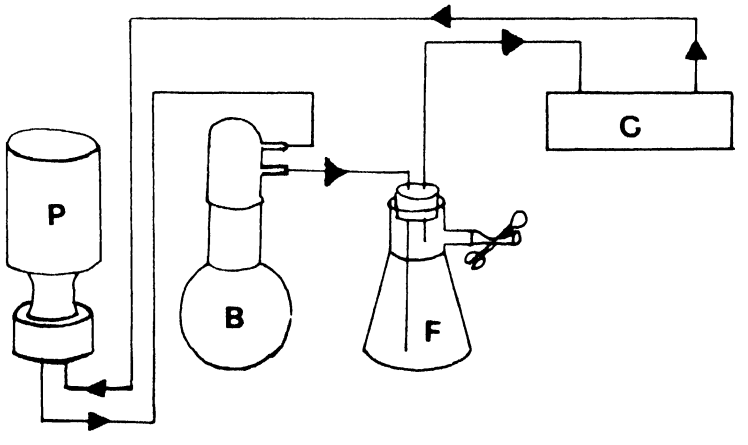


Fig. 28 Apparatus for the flameless determination of mercury
P: pump; B: boiling flask; F: filtering flask; C: absorption cell
(after Munns and Holland, 1971)

Reagents

a) Reducing solution: mix 50 ml H_2SO_4 with about 300 ml H_2O. After letting cool to room temperature dissolve 15 g NaCl, 15 g hydroxylamine sulfate, and 25 g $SnCl_2$ in the diluted H_2SO_4 and bring the volume to 500 ml with H_2O.

b) Diluting solution: add 58 ml HNO_3 and 67 ml H_2SO_4 to a volumetric flask (1 liter) containing about 500 ml H_2O and bring up to volume with H_2O.

c) Drying agent to be placed into filtering flasks (see Fig. 28): $Mg(ClO_4)_2$

Caution (Mg-perclorate may explode, if it comes into contact with organic substances)

d) Hg-stock solution for preparing standards: dissolve 0.1354 g $HgCl_2$ in 100 ml volumetric flask and bring up to volume with H_2O.

Hg-standards

Prepare internal standards by spiking the subsamples taken from a digested sample of the same matrix or prepare a model matrix taking into consideration the chemical composition of marine organisms (see Tab. III) and the digestion procedure used. Spike the standards with aliquotes from the Hg stock solution.

Procedure:

1) Transfer cold digested sample into a volumetric flask of appropriate size (e.g. 50 or 100 ml) rinse the digestion vessel with small amounts of distilled water, add to the volumetric flask and bring with H_2O to volume.

2) Transfer an aliquote (e.g. 20 ml) into the reaction vessel.

3) Adjust the output of the pump to circa 2 litres/minute.

4) Connect aeration-reduction apparatus as shown in Fig. 28 except for the gas inlet adapter.

5) Zero the AA spectrophotometer,

6) Add 20 ml of reducing solution and immediately connect the gas inlet adapter. Aerate for about three minutes and record the signal. Adjust the aeration time to obtain an optimal signal.

7) Disconnect pressure hose on the outlet of the pump and open vent on the filtering flask to flush the system.

The system is now ready for the next determination or for the calibration with standards. From the calibration curve and the dilution of the original sample calculate the Hg concentration in the sample.

6.4.1.1.2 Flameless Hg-determination in an open system (Parker, 1972)

The apparatus for determining mercury by flameless atomic absorption (Fig. 29) consists of an absorption tube (Vycor with Vitreosil end windows), tube holder, six reaction vessels, stand and stopper. Incorporated within the stopper is the air by-pass system which permits approximately 20% of the carrier gas to by-pass the reaction vessel. The balancing of the air flows between by-pass and reaction vessel is achieved through two orifices, one of which is the tip of the bubbler. Note that changing the size of this orifice may result in reduced sensitivity or ineffectiveness of the by-pass.

The by-pass is included to ensure that air entering the absorption tube is not saturated with water vapor. This prevents condensation on the end windows of the tube, which would result in a prolonged absorption signal with increased non-atomic component. A drying tube is not required when this system is used.

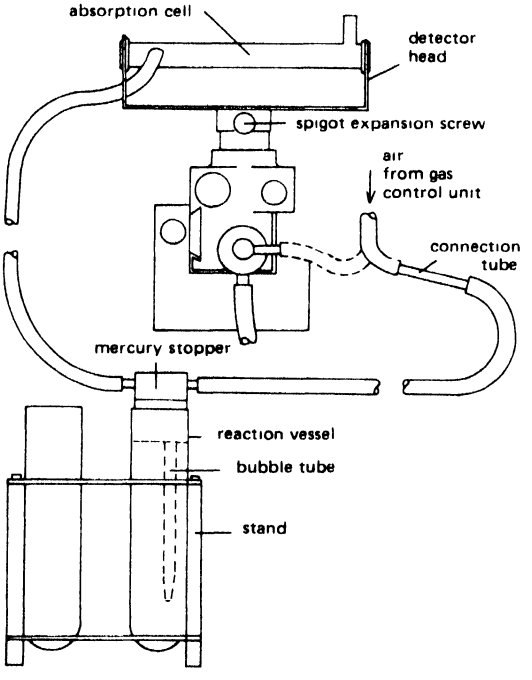


Fig. 29 General arrangement of Hg kit (Parker, 1972)
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Parameters Affecting the Signal

Stirring

The effectiveness of the stirring operation is the most important factor in obtaining good reproducibility and sensitivity.

As the solution is stirred, mercury diffuses from the liquid into the air space in the reaction vessel. The importance of proper stirring lies in the fact that the height of the recorded peak is entirely dependent on the concentration of mercury in the air phase. Consequently, it is important that the mercury content of the air should be consistently representative of the mercury content of the sample. This can only be achieved by ensuring that the mercury distribution between the air and liquid phases is in equilibrium. If the stirring is not sufficiently vigorous, or the time allowed is too short, the required equilibrium will not be achieved and reproducibility and sensitivity will suffer accordingly.

It is recommended that stirring be sufficiently vigorous to produce a vortex which descends to the bottom of the reaction vessel. Under these conditions the distribution is near equilibrium after 60 seconds, the concentration of mercury in the gas phase changes only slowly, and acceptable results are obtained.

Gas Flow Rate

Under normal circumstances, large changes in flow rate result in very small changes in peak height. This is because the peak height depends on the concentration of mercury in the air, and this is not greatly affected by the rate of purging through the cells.

A flow rate of 4 litres/minute will result in a fast, sharp peak, and is recommended for normal use. If the response time of the recorder or electronics system is greater than approximately one second for full scale deflection, curved calibration and some reduction in accuracy at higher absorbances will result. These problems can be overcome without compromise by reducing the air flow rate - for example use 1.5 - 2 litres/minute. The residence time of the mercury-laden air in the light path is increased and the peak is recorded more slowly.

Bubbling Through or Over the Solution

Provided that the solution is thoroughly stirred, and equilibrium distribution of mercury between the phases has been achieved, the purge gas may be passed over or through the solution. The same result is obtained by each method. Advantages of not passing the purge gas through the solution are that very little water vapor is carried to the absorption tube and foaming of samples is prevented. It is possible to pass the purge gas over the surface of even large samples by continuing the stirring while purging.

Apparatus

a) AA spectrophotometer with Hg lamp, continuous spectrum lamp and pen recorder.

b) Varian Hg determination kit or similar (Fig. 29).

c) Two washing bottles to eliminate Hg from the air (see above).

Reagents

Reducing solution: Dissolve 10 g hydroxylamine sulphate and 15 g $SnCl_2 \cdot H_2O$ in 40 ml 1:6 H_2SO_4 and bring to 100 ml with H_2O.

Hg- Standard

Prepare Hg standards as described in the previous method.

Procedure

1) After assembling the kit following the instruction supplied by the manufacturer, set the air flow to the desired value consulting the remarks made about the gas flow above. Turn off at the 'T' valve.

2) Transfer the cold digested sample into a volumetric flask of convenient size (50, 100 ml) with distilled water and bring to volume.

3) Add 5 ml of diluted digestion solution and 5 ml of distilled water to a reaction vessel.

4) Add 1 ml of reducing solution and insert immediately the stopper.

5) Stir for 90 seconds and measure the peak height after turning the air on by means of the 'T' valve.

6) Remove reaction vessel after turning off the air.

The apparatus is now ready for a new determination; for the calibration curve and the dilution of the original sample calculate the Hg content.

6.4.1.2 Determination on inorganic and total Hg without wet combustion

The following methods have not found wide application and, therefore, difficulties may arise. Since, however, these methods will allow the determination of organic mercurials with AAS, the detailed procedure is given below. Yield determinations are to be carried out before using them for routine analysis.

The methods are based on the observation that inorganic Hg can be determined in the presence of organic Hg after complexing the inorganic Hg with cysteine in an acid medium from which the inorganic Hg complexed with cystein can be released after addition of $SnCl_2$ and NaOH to make the solution alkaline (Magos, 1971). Total Hg can be determined by adding either Cd or Cu salts during the reduction step which will cause the release of Hg from both inorganic and organic compounds.

Baltisberger and Knudson (1974) have utilized the part of the method involving the selective liberation of inorganic Hg by tin (II) sulfate in sulfuric acid medium in the presence of organic Hg compounds to determine Hg in fresh water. The Hg_T was determined after treatment with H_2O_2.

Methodology according to Magos (1971)

Apparatus

AA spectrometer complete with kit for reduction-aeration method.

Reagents

a) Cysteine hydrochloride, 1% (w/v) high purity

b) NaCl 1% high purity

c) H_2SC_2 16 N high purity

d) $SnCl_2$, for each determination 100 mg are needed

e) $SnCl_2$ - $CdCl_2$ reagent: Add 25 g of $SnCl_2$ and 5 g of $CdCl_2$ to about 40 ml of distilled water, heat until boiling. Bring to 50 ml volume in a volumetric flask after cooling.

f) NaOH 45% high purity

g) Silicone MS antifoam: Occasionally this material had to be employed to avoid foaming of the material in the reaction vessel.

Hg standards

a) Inorganic standard: Dissolve 0.6767 g $HgCl_2$ in 5% H_2SO_4 and bring it with the H_2SO_4 to 1000 ml in a volumetric flask. Add 1 ml of this solution, 9 g NaCl, 0.7545 Na-EDTA and 0.063 g L-cysteine hydrochloride to a 1000 ml volumetric flask, dissolve and bring up to volume (In the refrigerator this solution remains unchanged for at least six months).

b) Methylmercury standard: Dissolve 60.08 mg methylmercury chloride (e.g. AB Casco, Stockholm, Sweden) in a 100 ml volumetric flask in acetone and bring up to volume. Make from this solution a 1 : 1000 dilution in a volumetric flask with distilled water.

In an alternative way the standards can be prepared from 36.96 mg of methylmercury dicyandiamide (e.g. AB Casco, Stockholm, Sweden) with distilled water; bring it first to 500 ml in a volumetric flask and then dilute 1 : 100. These solutions are not very stable and the Hg content must be checked, since losses occur due to volatilization and precipitatio

Sample preparation

Magos (1971) proposed a sample preparation which is a similar procedure to the one suggested by Skare (1972). Skare found that fish meat could be easily homogenized, if it were dissolved in excess alkaline with occasional shaking and standing overnight at room temperature. This homogenate was stable for a long time, if stored in a refrigerator.

The samples may be homogenized in a 1% NaCl solution with a stainless steel, glass or Teflon homogenizer. In many cases the following procedure may be used which is simpler, but it presents contamination hazards. The relative high concentration of NaOH will dissolve the glass surface in contact with the NaOH and the impurities contained in the glass will be released (Adams, 1972).

Fishmeal: 0.5 g are mixed with 1 ml of the 1% cysteine solution, 1 ml of 20% NaCl solution and 1 ml of the 45% NaOH solution. Heat to boiling point and transfer the solution with distilled water into the reaction vessel of the Hg apparatus.

Whole specimen: Bring a 40% NaOH solution of twice the weight in g of the specimen in ml to boil. Drop the specimen into the NaOH and let it dissolve (20 minutes). After boiling dilute with distilled water so that the final NaOH concentration will be 20% w/v based on the weight of the specimen.

a) Determination of inorganic Hg

Procedure :

1) Prepare instrument according to the method chosen.

2) Transfer with a pipette an aliquot of the sample (1 - 20 ml) or of the standard into the reaction vessel.

3) Add 1 ml of the cysteine solution and make up to 21 - 23 ml with the 1% NaCl solution.

4) Add 10 ml of 15 N H_2SO_4

5) Add 100 mg of $SnCl_2$ to the reaction vessel and 20 ml of the 45% NaOH quickly and close the reaction vessel immediately.

6) Record the peak height according to the reduction-aeration method used.

b) Determination of total Hg

Procedure:

Proceed as above except for step 5 as follows:

5) Add 1 ml of the $SnCl_2$ - $CdCl_2$ reagent (Reagent 'e')

c) Determination of first inorganic and then organic Hg in the same reaction vessel

Follow procedure (b) until step 6. One to three minutes after the addition of the NaOH add 10 ml of 15 N H_2SO_4 and 1 ml of the $SnCl_2$ - $CdCl_2$ reagent and 20 ml of 45% NaOH and read the peak height.

6.4.2 Determination of cadmium

Since the lower typical concentration of Cd in marine organisms is near the DL (Tab. IV) flame techniques, as recommended by the manufacturer of the instrument, should only be employed for the higher concentrations.

Should lower concentrations be encountered, the recommendations of the Analytical Methods Committee (1975) of the Chemical Society could be employed. This included an extraction with a liquid ion-exchanger (Amberlite LA-2) in 4-methylpentan-2-one after wet digestion and transformation of the Cd into iodocadmate. Julshamn and Braekkan (1975) and Childs and Gaffke (1974) concentrated the trace metals Cd, Fe, Cu, Mn, Zn and Pb after complexing with Na-diethyl-dithiocarbamate and extraction with methyl-isobutyl after wet digestion. In this way they could determine approximately 0.05 µg Cd/g FW.

If a graphite furnace is available its lower DL (Tab. IV) will allow analyses without any additional concentration processes. Details on the use of the graphite furnace can be obtained consulting the manufacturers instructions and the papers of Sperling (1975) and Slavin et al. (1975).

6.4.3 Determination of total arsenic

Arsenic is one of the 'difficult' elements to determine with AAS because its absorption lines are both below 200 nm and direct aspiration of arsenic containing solutions into the flame suffer from high background absorbance and noise level resulting in a low signal to noise ratio. Several remedies have been proposed such as the use of different flame combustion mixtures and burner constructions (Kirkbright et al., 1969, Kahn and Schallis, 1968), but many of the problems can be eliminated when gaseous arsine is generated from the sample and analysed with an argon-hydrogen flame.

Outline of the determination of As by arsine generation:

After wet digestion of the organic substance with nitric acid or nitric acid mixture the solution is purged of the interfering nitrogen oxides with CO_2 or by boiling. Then the sample is diluted with a mixture of HCl and H_2SO_4. The arsenic present is reduced to As^{+3} with KI and $SnCl_2$. Finally the arsine gas is produced by the addition of zinc powder, and the arsine gas transported with argon into the flame, where the arsine is atomized. Several different arsine generation systems have been proposed and later improved, ranging from collecting As in a liquid nitrogen cold trap to collecting the arsine in a rubber balloon before releasing the accumulated gasses into the AAS flame. (Holak, 1969, Manning, 1971, Dalton and Malanoski, 1971, Thompson and Thomerson, 1974, Freeman and Uthe, 1974). Only a few As determinations employing AAS have been carried out on marine organisms (e.g. Windom, 1972).

An investigation on the efficiency of different reductants showed that the strongest absorption signal was obtained when KI, $SnCl_2$ and zinc powder was used (Maruta and Sudoh, 1975). Within a certain range the amount of the KI and $SnCl_2$ added is not critical (Maruta and Sudoh, 1975). Vijan and Wood (1974) have published an automated method which works without a flame and sweeps the generated hydrides of As, Sb, Bi, Se, Te and Ge into a heated, windowless quartz cell, using a proportional pump. Maruta and Sudoh (1975) studied several factors which may interfer with the determination. They conclude that serious interferences arise only from nitric acid, lead, chromium and selenium, while solutions of arsenic containing 0.05 - 1 M sulfonic, perchloric or phosphoric acid in the presence of 1 M HCl did not interfer. If HNO_3 was used for digestion of biological samples all interfering oxides of nitrogen can be eliminated by boiling the samples prior to the arsine generation. Less than 5% change of the signal was observed after addition of 80 µg of Na, K, Mg, Ca, Mn, Co, Ni, Cu, Al, Fe, and V to a sample solution containing 0.8 µg of As. Under these conditions Cr, Pb and Se reduced the signal by 30%, 13% and 10% respectively. Cr and Pb interfered in the generation of arsine, but not in the atomization process, while Se reduced the signal after it was introduced into the flame following formation of H_2Se. Adding 8 µg of Cr, Pb or Se did not interfer.

Stux and Parker (1972) observed that the zinc powder used may contain high As impurities resulting in high blank values. It is, therefore, necessary to test several batches of zinc from different suppliers in order to select a zinc powder with a low As concentration.

All of the above mentioned methods with the exception of Maruta and Sudoh's accumulate the arsine generated over a certain time period and transfers after the generation, the arsine in a very short time interval into the flame. Stux and Parker (1972) have studied the parameters involved in the arsine generation in order to eliminate these holding devices. Nearly all firms which manufacture AAS instruments offer kits for the determination of As and Se. Their instructions should be followed when applying their kits. For an illustration, the methodology of Stux and Parker (1972) are given here.

Apparatus

Varian Techtron atomic absorption spectrophotometer with hollow cathode lamp for As and hydrogen continuum lamp or similar apparatus.

Burner for nitrogen-hydrogen entrained air flame.

As and Se kit (Fig. 30).

Reagents

a) hydrochloric acid, high purity

b) sulphuric acid, high purity

c) 15% KI solution, high purity

d) 20% $SnCl_2$ in concentrated HCl, high purity

e) As - free zinc powder

Procedure

If nitric acid has been used in the wet digestion, it must be removed either by boiling under a fume hood or by purging the sample with CO_2.

Using a nitrogen-hydrogen-entrained air flame with a nitrogen flow of 7.5 units (approximately 11 litres/minute), optimize the burner position and flame while aspirating an aqueous arsinic solution. Note that the optimum conditions obtained are also suitable for selenium determinations. Remove the nebulizer and nebulizer bung from the spray chamber and fit a plain inlet bung equipped with a barb type hose connector which will accept 1/4 inch laboratory tubing. Connect the special stopper as shown in Figure 32 and set the control valve at "BY-PASS".

Adjust the support pressure to approximately 15 psi to give a nitrogen flow of 5.5 units (8 litres/minute). Now use the auxiliary support control to give a nitrogen flow of 7.5 units (11 litres/minute). Ensure that the hydrogen flow remains at the optimum rate established when originally setting the flame.

For the analysis, 20 ml of sample solution, which should be 20% in hydrochloric acid and 5% in sulphuric acid and contain up to 250 ng arsenic is placed in the reaction vessel. Add 1.0 ml of 15% potassium iodide solution, and 1.0 ml of 20% stannous chloride in concentrated hydrochloric acid. Insert the special stopper in the reaction vessel, switch the valve to SAMPLE and record the air peak. When the pen has returned to zero, rapidly inject 2.0 ml of a zinc slurry (1 g/ml) through the septum. A vigorous reaction will now occur and the peak will appear within 1-2 seconds. When the recorder pen has returned almost to the baseline, switch the valve back to "BY-PASS" and immediately remove the stopper from the reaction vessel. The system is now ready for the next sample.

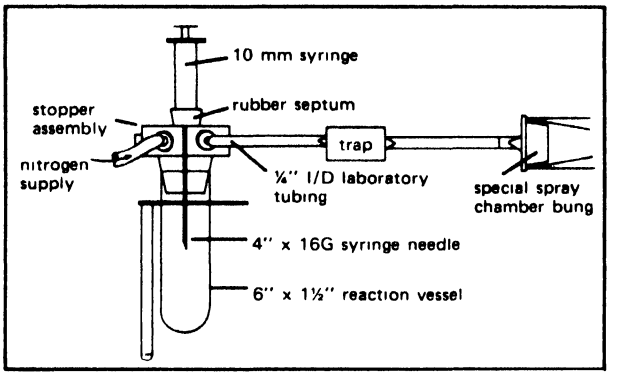

Fig. 30 Apparatus for the As and Se determination (Stux and Parker, 1972)
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6.4.4 Determination of total selenium

Selenium can be determined with the same kit which is used for the As determinations (see section 6.4.3) following with a slight modification the same procedure. However, it has been observed that when reducing Se (II), (IV) or (VI) with KI and $SnCl_2$, once the element was formed, it rapidly changed into a more stable form which was not readily available for further reduction (Stux and Parker, 1972). Stux and Parker overcame this difficulty by increasing the acid strength to 40% HCl and 10% sulfuric acid. Thompson and Thomerson (1974) observed that solutions of Se (VI) gave negligible response in comparison with equivalent amounts of Se (IV). Treating the samples solutions with 'aqua regia' did improve the Se (VI) detection but the efficiency was still less than 50%. It seems, therefore, that if the valence state of Se (VI) occurs analytical problems may be encountered.

6.4.5 Determination of total copper

Concentration of copper in marine organisms range between 200 and 50000 µg Cu/kg FW (Tab. III). Since the DLs for Cu with flame AAS are about 0.01 µg Cu/ml for the most sensitive line 324.7 nm, diluting or selecting a less sensitive line, e.g. 222.6 or 244.2 may bring the concentration into the optimal range (Tab. IV). Topping (1973), Windom et al. (1973) and Segar et al. (1971) among others have used AAS for the determination of Cu in marine organisms without reporting difficulties. In the ICES intercalibration exercise the results of the Cu-determination were in very good agreement indicating that no difficulties occurred. If concentrations are to be determined which are smaller than the DLs, Cu may be extracted with ammonium pyrrolidine-dithiocarbamate (Analytical Methods Committee, 1971) or with sodium diethyl dithiocarbamate (Julshamn and Braekkan, 1975). If a graphite furnace is available its lower DLs will make extraction unnecessary. Indications on the operating conditions can be found in the operation instructions and by Slavin et al. (1975).

6.4.6 Determination of total zinc

Due to the high zinc concentrations in marine organisms and the low DLs of the flame AAS no difficulties are anticipated when the digested samples are analysed under the standard conditions suggested by the manufacturer of the AAS instrument (Windom et al., 1973; Topping, 1973; Cutshall and Holton, 1972; ICES, 1974). Dilutions may be necessary in order to bring the high concentrations into the optimal range (Tab. IV), or about 1000-times less sensitive 307.6 line may be used avoiding zinc contamination with the diluting solution.

If on the other hand, very small zinc concentrations have to be determined, extraction of zinc with ammonium pyrrolidine-dithiocarbate may be employed (Analytical Methods Committee, 1973), or, if available, a graphite furnace can be used (Clark et al., 1973).

6.4.7 Determination of total manganese

DLs are low enough to allow direct determination with flame AAS. Bradfield (1974) studied the possible interferences in the determination of plant materials. He observed interference of Ca and Mg on the absorbance of Mn in the presence of sulfate. No interferences were observed from Fe, K and Cl. The addition of Lanthanium chloride or NaCl (1 mg/ml) reduced the interference practically to zero. Other remedies are: measure the absorbance higher in the flame, decrease the droplet size and hence the size of solid particles in the flame with the addition on an alcohol. Bradfield suggests, therefore, to avoid wet combustion with H_2SO_4. At any rate these interferences can be controlled by using the standard addition method in subsamples of the original sample in order to match the matrix adequately. Bradfield's remedies may be used if the sensitivity is insufficient for the Mn determination.

Shigematsu et al. (1975) studied optimal conditions of temperature, different gases, etc. for the Mn determination with a graphite atomizer. No decrease of absorbance was observed from HCl, HNO_3, $HClO_4$ and H_2SO_4 at low concentrations (0.05 M) on 25 µg Mn/l. Only H_3PO_4 reduced the signal by 15%. The effect of salt at 40 times the Mn concentration was neglectable except for Na_2SiO_3. At 400 and 4000 times, however, the signal was significantly reduced by $CaCl_2$ (48 and 77% respectively) showing the same interference already mentioned above. Reduction of the Mn absorbance is to be expected, since marine organisms contain relatively large amounts of Ca and Mg and also of SO_4 in relation to trace elements (Tab. III).

6.4.8 Determination of total chromium

The determination of chromium (e.g. Slavin et al., 1975) should not present problems with respect to DLs, since the typical concentration of Cr in marine organisms is about 25 times higher (Tab. III). However, matrix effects are to be anticipated and, this has therefore to be taken into consideration, during the preparation of standards. Yanagisawa et al. (1970) report small interferences of Cu, Fe, Na, K, Zn and others in concentrations 5 times that of Cr (250 µg/ml). The temperature of the flame contributes considerably to the magnitude of the effects observed. Green (1975) demonstrated the importance of the valence state of Cr in perchloric acid solutions. He suggests that all Cr be converted into Cr (III) by a hydrogen peroxide treatment. Furthermore, an addition of NH_4Cl can increase the response by up to 30%. If standard determination with the air acetylene flames are not sensitive enough, extraction with ammonium pyrrolidine dithiocarbamate into methyl isobutyl ketone may be employed (Gilbert and Clay, 1973).

6.4.9 Determination of total lead

From a strictly analytical point of view, Pb determinations should not present special problems. The DLs are low enough to allow a direct analysis with flame AAS. However, severe contamination problems are to be anticipated, since Pb is ubiquitously present in air, water, on glass surfaces, etc. In fact, Patterson (1974), a specialist on Pb determination, maintained only recently that most Pb determinations are not valid because of Pb contamination. It is obvious, therefore, that much effort during the sample treatment and analyses has to be made to prevent contamination of Pb. Reagent blanks must be carefully checked and the air used in the AAS should be passed through washing bottles, especially if the laboratory is located in areas with intensive car traffic.

Interference of nitric acid, sulfuric acid and tin in the digestion of samples has been reported (Roschnik, 1973) thus, nitric acid digestions should be avoided or after digestion the nitric acid must be eliminated by boiling.

If the standard flame technique is not sufficiently sensitive, a preconcentration with ammonium pyrrolidine dithiocarbamate (Analytical Methods Committee, 1975a) or with diethylammonium diethyldithiocarbamate (Roschnik, 1973) may be applied. If a graphite furnace is available, its higher sensitivity may be of advantage (see Table III). Some indications on the programming of the furnace can be obtained by consulting the manufacturer's manual and the paper by Slavin et al., 1975.

6.5 Gas Chromatographic Analysis of Chlorinated Hydrocarbons

Gas-liquid chromatography (GLC) uses the differences in partition coefficients of various components of a mixture between a liquid stationary phase and a moving gas phase for separating the different components. The liquid phase is adsorbed on inert material. The inert material impregnated with the liquid phase is then placed in a glass, metal or plastic tube (chromatographic column) through which the "carrier gas" is passed. For analysis a sample containing analyte(s) is volatilized at an elevated temperature in the injection lock located at one end of the chromatographic column which in maintained at a given temperature in an oven (Isothermic GLC). The carrier gas (e.g. nitrogen) passing transports the sample through the column, causing a continuous dissolution of the analytes in the liquid phase along the tube and their continuous eluation from the liquid phase. Analytes which are less soluble in the liquid phase will be eluated before those which are more soluble, allowing a chromatographic separation of the different components of a sample. When the analytes leave the column they enter a suitable detector - in the Chl-HC analyses usually an electron capture detector (ECD) - which records eluation curves on a recorder (chromatogram). The amount of analyte is quantified from the peak area or, under certain conditions, from the peak height. Although GLC is a very sensitive technique, both identification and quantification raise several problems (Jensen et al., 1973; Chau and Sampson, 1975). Known mixtures of analytes can, in most cases, securely identified and quantified by a comparison with standards and by their retention times relative to a reference substance (e.g. DDE, aldrine, etc.), but, in environmental samples, the nature of the substances cannot always be predicted. Even when predictions can be made, different substances may possess the same retention time. For instance, the peaks of p,p'-DDE and p,p'-DDD coincide with the PCB peaks number 4 and 9 respectively, while p,p-DDT lies very near to the PCB peak number 10 (Figs. 31a and 31b).

As a remedy, the different analytes can be separated by solvent partition, column chromatography, thin-layer chromatography, selective destruction during pre-treatment, etc. Repetition of the gas chromatographic analysis after chemical transformation can also be used for identification.

In recent years, a characterization by mass spectrometry has been increasingly employed to supply additional information on the analytes examined. The mass spectrometric analysis consists in bombarding the analyte with an electron beam which causes electron loss and

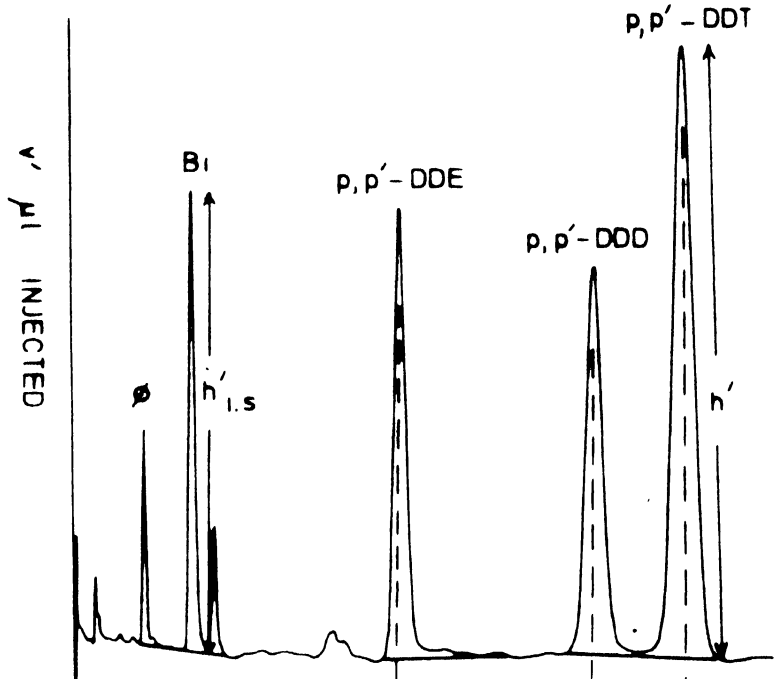

Fig. 31a

Chromatogram of DDT standard

The Standard contains:

p,p´- DDE	0.1 ng/µl
p,p´- DDD	0.2 ng/µl
p,p´- DDT	0.3 ng/µl

(from Jensen et al., 1975a)

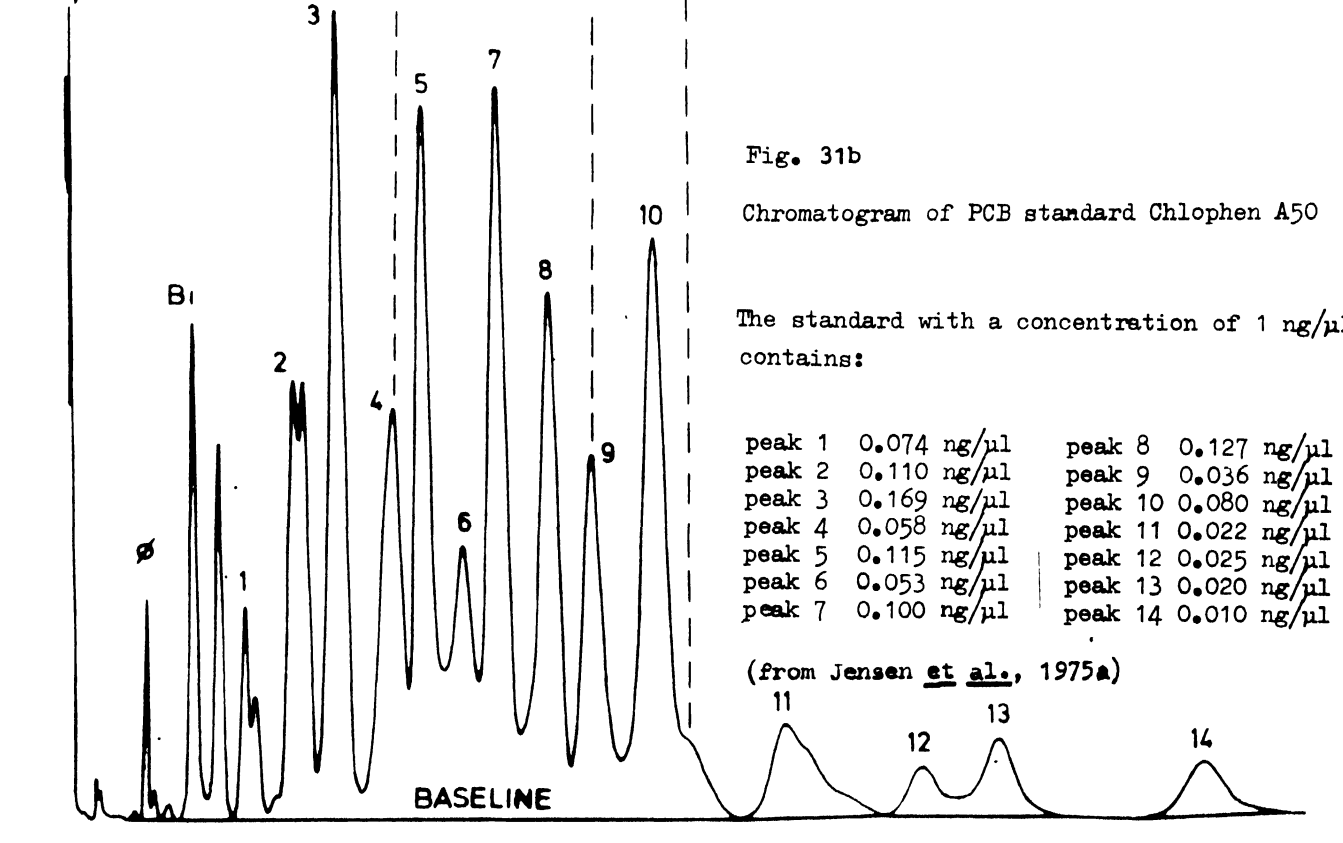

Fig. 31b

Chromatogram of PCB standard Chlophen A50

The standard with a concentration of 1 ng/µl contains:

peak 1	0.074 ng/µl	peak 8	0.127 ng/µl
peak 2	0.110 ng/µl	peak 9	0.036 ng/µl
peak 3	0.169 ng/µl	peak 10	0.080 ng/µl
peak 4	0.058 ng/µl	peak 11	0.022 ng/µl
peak 5	0.115 ng/µl	peak 12	0.025 ng/µl
peak 6	0.053 ng/µl	peak 13	0.020 ng/µl
peak 7	0.100 ng/µl	peak 14	0.010 ng/µl

(from Jensen et al., 1975a)

fragmentation of the molecule. The fragmentation and the pattern of the spectra of the different fragments resulting from bond fission and rearrangement of atoms are highly characteristic of the original molecular structure and can, therefore, be used for an identification of the unknown analyte. This can be done either simultaneously with the gas chromatographic analysis or in a batch treatment, i.e. after collection of the fractions eluated from the gas chromatograph.

In combined gas chromatography/mass spectrometry, the gas chromatograph is coupled directly with a mass spectrograph, allowing the continuous introduction of the eluated compounds into the mass spectrograph which, during the registration of the eluation curve, scans the mass spectrum of the fragments repeatedly. The mass spectrum obtained is analysed by comparing it with the spectra of known substances either manually or with the aid of a computer (Stenhagen et al., 1973). More details may be obtained from reviews by Biros (1971) and Fenselau (1974). The major drawback of Chl-HC identification with a mass spectrograph is its relative high instrument cost as compared with that of a gas chromatograph and the need for additional specialized personnel for its operation. This will make it mandatory for most participating centres to rely on chemical separation and transformation for analyte identification.

For the purpose of the pilot projects, the screening will be limited to certain persistent pesticides which will allow the use of much simpler procedures, especially during the pre-treatment of the samples.

In a recent review on the aspects of organic marine pollution, Duursma and Marchand (1974) point out that non-persistent pesticides are very unlikely to contaminate significantly the marine environment since they will be degraded before reaching it. In fact, only the PCBs and pesticides such as dieldrin, endrine, the DDTs, heptachlor, aldrin, lindane, chlordane, toxaphene, hexachlorocyclohexane, endosulfan, methoxychlor and wastes from the PVC production (aliphatic Chl-HCs) have been detected in marine organisms (National Academy of Sciences, 1973).

Before Chl-HCs can be analysed in a gas chromatograph, interfering organic substances, specially lipids, must be eliminated in a pretreatment. For a complete analysis, rather complicated pretreatments are necessary. Several multi-residue procedures have been developed which are described in detail in publications such as the Pesticide Analytical Manual (U.S. Department of Health and Education, 1975), the EPA Pesticide Manual (Thompson, 1974), and the Official Methods of the AOAC (Horwitz, 1975). The Pesticide Analytical Manual will be supplied to all participants carrying out Chl-HC analyses thanks to the courtesy of the U.S. Food and Drug Administration.

Somers (1974) reviewed recently pesticide analyses of various foods, including fish, for the FAO/WHO Expert Consultation to Identify the Food Contaminants to be Monitored and to Recommend Sampling Plans and Methodology (Rome, 7-11 October 1974). This review may be consulted for a comparison of the different methodologies used in pesticide analyses in different methodologies used in pesticide analyses in different countries.

It should also be mentioned that the extraction of large environmental samples is expensive, not only in work time, but also because pesticide grade solvents are costly. Whenever possible, small sub-samples should be studied and miniature equipment used, such as micro-blenders and micro-soxhlets (from Fisher Scientific or Karl Kolb Scientific Technical Supply, Buchschlag-Frankfurt, Federal Republic of Germany).

The above-mentioned manual considers that a screening for all possible pesticides, and hence their methodology, can often be simplified. For example, if the monitoring is limited to the PCBs, DDTs and lindane (γ BHC), a simple clean-up after lipid extraction with concentrated H_2SO_4 is sufficient. If, however, dieldrin (a first priority Chl-HC) is also to be monitored, a more complicated pre-treatment is necessary. Since during complicated sample preparation procedures loss and contamination occur, sample preparation should be kept to a

minimum and yield determinations are essential. The above-mentioned simple acid clean-up may also be used to determine separately the PCBs, DDTs and lindane, thus allowing a check on complicated pre-treatment.

6.5.1 Cleaning of glassware, instruments and reagents

Detailed instructions on how to carry out the cleaning and avoid contamination of various origins are given in the Pesticide Analytical Manual, and should be followed scrupulously. Giam and Wong (1972) investigated, especially, the problems of background contamination in the analysis of open ocean biota. They recommended to heat all materials which can withstand a high temperature (glassware, glasswool, aluminium foils, Florisil, sodium sulfate and sodium chloride, etc.) in an oven at 300-350°C overnight. All other materials, including blenders, which are not heat resistent can be cleaned by solvent extration, first with acetone and then with petroleum ether or hexane. Distilled water should be distilled twice in the presence of 0.1-0.2 g of potassium permanganate for every 3 litres in a special distillation apparatus set aside for this purpose only.

6.5.2 Pre-treatment

Three steps can be identified in the pre-treatment:

(a) Extraction of the lipids (fat) plus Chl-HCs;

(b) determination of the amount of lipid (fat content)

(c) clean-up and separation of interfering substances.

Principle of the lipid extraction procedure

The tissue is first homogenized in a high speed blender in the presence of anhydrous Na_2SO_4. Then the homogenate is extracted in the same blender (cold extraction) or a Soxhlet extractor (warm extraction) several times with the solvent selected (e.g. petroleum ether hexane); the various extracts are combined, dried with anhydrous Na_2SO_4 and finally concentrated e.g. in a Kuderna-Danish concentrator.

Detailed procedures are given in the Pesticide Analytical Manual (section 211ff, especially 211, 13f), in Official Methods of AOAC (Horwitz, 1975) for Fish under procedure 29.012(e), in the EPA Pesticide Manual (Thompson, 1974) under section 5. A(1), and in Farrington et al. (1972).

Lipids have been extracted from fish and other aquatic organisms with hexane (e.g. Harvey et al., 1974; Addison et al., 1972; Murphy, 1972), with hexane/acetone (Bourne and Bogan, 1972), with petroleum ether (Nimmo et al., 1971, Pesticide Analytical Manual), with acetonitrile (Smith and Cole, 1970), with hot perchloric-acetic acid mixture (Stanley and Le Favoure, 1965) and with other solvents. The hot perchloric-acetic acid mixture used by Stanley and Le Favoure may be unsuitable because it destroys several "pesticides", for instance dieldrin, malathion, parathion, and reduce the recovery of aldrin, heptachlor, epoxide and endrin.

Extraction mixtures containing dehydrating solvents such as acetone, isopropanol, etc., make the cell membranes more permeable for lipids and thus increase the amount of fats extracted (Jensen et al., 1973).

According to the EPA Pesticide Manual, an extraction with petroleum ether should be preferred to other solvents because, so far, no collaborative study has been carried out to show that different extractions are really equivalent. In fact, recently, Hattula (1974) compared different cold column extractions and different hot Soxhlet extractions of Na_2SO_4 analysing dried fish homogenate of 3 fish species containing different fat contents. Four different solvents were employed: diethylether, diethylether and n-pentane (1:1), 25%

n-hexane in acetone and 10% diethylether in petroleum ether (1:1) and chloroform and methanol (1:1). Hattula could show that cold extractions yield, in general, lower (50-90%) amounts of extractable lipids than the hot Soxhlet extraction, but not in all cases. The most erratic results were obtained with "classical" chloroform/methanol extraction which extracted also proteinaceous material. Recalculating Hattula's data, it was found that the different methods will not even extract similar lipid fractions from fish of different fat content nor extract with the different solvents similar fractions of Chl-HCs from the three fish species studied.

Similar large differences in the analytical results were observed by Duursma (1976) when comparing 6 different methodologies for the determination of "pesticides" in aquatic organism and sediment samples.

Although it seems reasonable to expect that different solvent mixtures and methodologies would extract different lipid fractions, there are several publications which show that different methodologies yield similar results (e.g. Pettinati and Swift, 1975). However, these results were obtained by comparing homogeneous materials such as fish meal of similar fat content or different forms of meat. If, on the other hand, meat, vegetables and various composite diets with or without acid hydrolysis were used to compare different extraction methodologies, considerable differences in lipid yields are observed (Conway and Adams, 1975).

Recommended reference extraction methodology

In the light of these results, it would be advisable to use in the pilot studies only one type of solvent, i.e. petroleum ether (30-60°C), employing the blender method as described in the Pesticide Analytical Manual, section 211.13f. In very hot climates, however, hexane, which has a high boiling point, may be better. If other methodologies are being used or are favoured, it is highly recommended to compare them on each species with the petroleum-ether blender method in order to allow a comparison of the results from different laboratories.

Clean-up and separation of interfering substances

Multi-residual analyses: Clean-up and serations for multi-residual analyses are complicated. They rely on partitions between hexane and acetonitrile. Further purification and separation are achieved with chromatographic columns, such as Florisil, silica gel, alumina, magnesia, celite, etc., and combinations of these absorbents. Detailed procedures are given in section 211ff of the Pesticide Analytical Manual, the EPA Pesticide Manual, section 5.A, and in the Official Methods AOAC, under procedure 29.014-7.

Simplified pre-treatment for the analysis of DDTs, PCBs and lindane

Very simple clean-up and separation procedures can be employed if the analyses are restricted to the DDTs, PCBs and lindane. Murphy (1972) has shown that a simple treatment with concentrated H_2SO_4 after the extraction of the lipids is sufficient for the gas chromatographic analysis of these substances. Duursma (1976 and personal communication) suggests, after petroleum or hexane blender extraction (see Pesticide Analytical Manual, section 211.13f), three simple clean-ups for (A) acid stable Chl-HCs (PCBs, DDT + metabolites), BHC and 2,4 D-esters without and with pre-concentration, (B) for the acid-stable Chl-HCs plus aldrin, dieldrin and endrin, and (C) weak and strong alkaline saponification which will transform certain Chl-HCs by dehydrochlorination and thus may serve as a clean-up and as an identification at the same time (see below).

A. Clean-up for acid resistant Chl-HCs without extract pre-concentration

Procedure:

1. Add 8 drops of concentrated H_2SO_4 to 2 ml subsample of the extract contained in a small centrifuge tube.

2. Shake vigorously for 10 minutes on a vibrator and centrifuge.

3. Inject 10 µl from the surface layer into the GLC.

A'. Clean-up for acid-resistant Chl-HCs with extract pre-concentration (to be employed if the chromatographic response after the previous clean-up is too small).

Procedure:

1. Evaporate 10 ml of the original extract in a graduated centrifuge tube by placing the tube into a water bath of $\pm$ 40°C and blow clean air or N_2 through a pipette onto the surface of the extract until the volume is reduced to exactly 1 ml.

2. Add 4 drops of concentrated H_2SO_4, shake vigorously for 10 minutes on a vibrator and centrifuge.

3. Inject 10 µl of the supernatant hexane into the GLC.

B. Clean-up for acid stable Chl-HCs plus aldrin, dieldrin and endrin (Florisil chromatography)

Florisil eluation can be carried out with a hexane ethyl-ether mixture or a petroleum ethyl-ether mixture. The analyst should be aware that quantitative Florisil eluations are not easy to achieve and require considerable experience. Therefore, before treating unreplaceable actual samples, trial runs with yield determination using spare samples spiked with relevant Chl-HCs should be carried out.

6% ethyl-ether in petroleum ether will eluate:

Aldrin	Heptachlor epoxide	BHC
Lindane	DDD	PCBs
Heptachlor	DDE	Disyston
Methoxychlor	DDT	

15% ethyl-ether in petroleum ether:

Endrin	Parathion
Dieldrin	Methyl parathion
Thiodan I	Malathion (trace)

and 50% ethyl-ether in petroleum ether:

Thiodan II	Malathion

For the detailed procedure see the Pesticide Analytical Manual section 211.14d. For a hexane ethyl-ether eluation follow the following instructions (Duursma, personal communication):

Procedure:

1. Prepare a Florisil chromatographic column in a 12 cm long 6 mm diameter glass tube closed at one end with a sintered glass disc or glasswool cleaned by high temperature heating by adding pre-conditioned Florisil (see Pesticide

Analytical Manual section 121.3 and 211.14d) so that a 2 cm long column results; place a small centrifuge tube under the column.

2. Pour 2 ml of the extract through the column and collect in the centrifuge tube.

3. Add subsequently 8 ml of a hexane/diethyl-ether mixture (9/1; v/v) and collect always in the same column. Evaporate; eluate to 2 ml exactly as described under A'1.

4. Inject 10 µl into GLC. Chromatogram will include dieldrin, aldrin and endrin, if present.

5. Follow procedure A1-A3 and the H_2SO_4 will destroy all Chl-HCs except the acid-resistant ones. Note that aldrin will not be completely destroyed.

6. Inject 10 µl into GLC for acid-resistant Chl-HCs and compare the two chromatograms obtained.

C. Weak and strong alkaline saponification

Often peaks of different Chl-HCs, especially the ones of PCBs, overlap with those of the DDTs (see Figure 31). A saponification with alcoholic KOH can serve as a clean-up since the fats will be hydrolysed and, at the same time, dechlorinates certain DDTs, for instance pp'DDT is transformed into pp'DDE and pp'TDE into pp'MDE, etc., while the stable PCBs will resist this treatment unchanged. This allows the employment of alkaline saponification, both as a confirmation test for the PCBs and an elimination of interference in the quantitative determination of the PCBs. The Pesticide Analytical Manual procedure is described under section 211.15d and also mentioned under 251.16(1).

Duursma (personal communication) suggests the following procedure:

Weak saponification:

1. To 2 ml hexane extract in a small centrifuge tube add 1 pellet of KOH dissolved in 0.2 ml of distilled water.

2. Shake slightly, centrifuge and inject 10 µl of the hexane supernatant into the GLC. The chromatogram represents a weak saponification.

3. Wait 6-24 hours, and inject again 10 µl into the GLC. Compare the peaks of the two chromatograms obtained.

Strong saponification:

1. To 2 ml of extract in a small centrifuge tube add 1 pellet of KOH dissolved in 1 ml of ethyl-alcohol (ca. 2% alcoholic KOH).

2. Shake for 10 minutes and inject the hexane supernatant.

3. Compare chromatograms obtained with those obtained through the other procedures.

The effect of alcoholic saponification is illustrated in Figure 32. A precise clean-up for different matrixes is difficult to prescribe and therefore the above procedures are to be modified according to the analyst's experience. Spiking sub-samples with the various Chl-HCs expected and running them through the whole procedure is recommended to increase accuracy in the interpretation of the chromatograms.

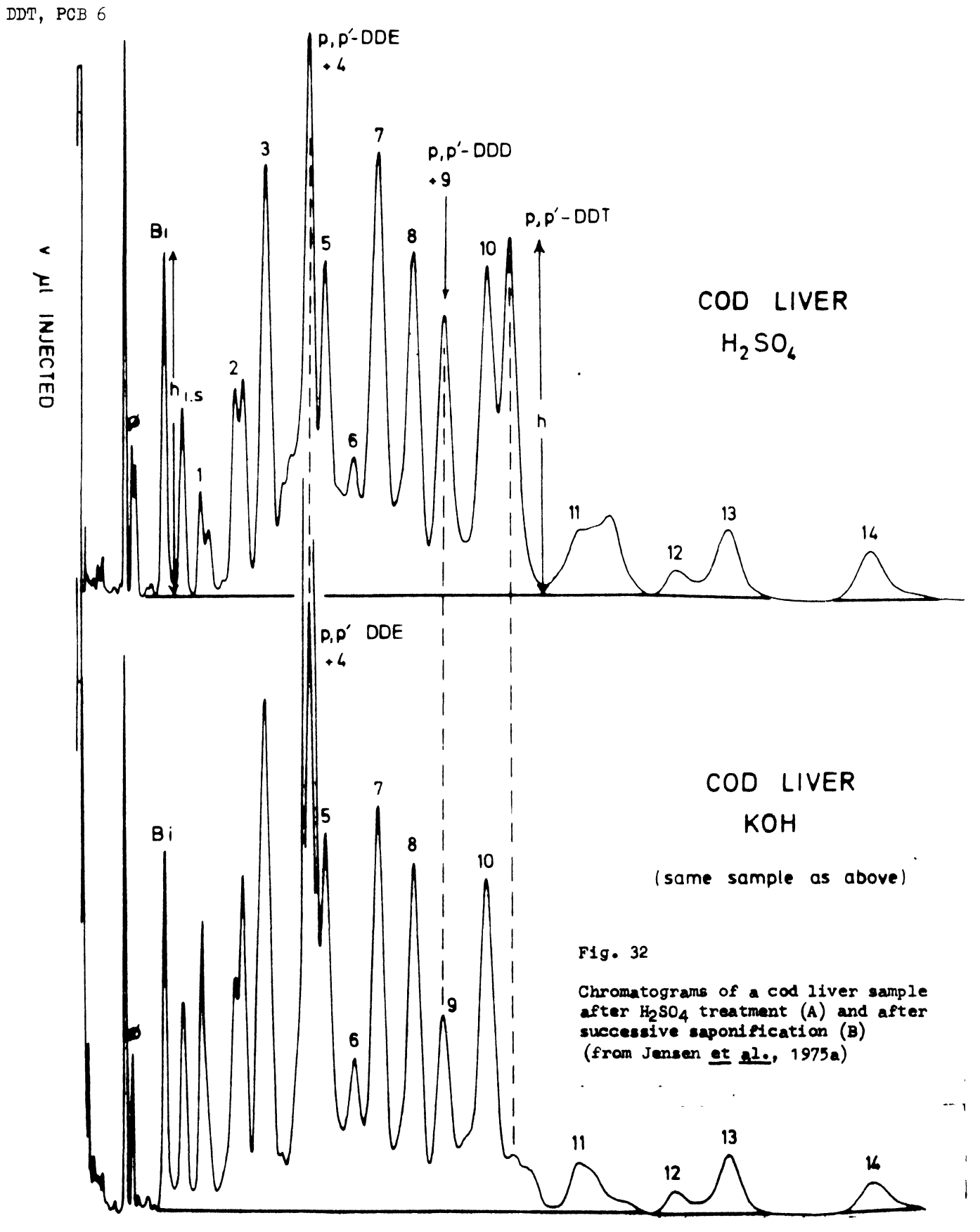

Fig. 32

Chromatograms of a cod liver sample after H_2SO_4 treatment (A) and after successive saponification (B) (from Jensen et al., 1975a)

Quantification of chromatograms

The different methods and various aspects of quantification of the chromatograms are discussed in the Pesticide Analytical Manual, section 302.4ff. Special difficulties arise in the PCB analysis (Jensen et al., 1973). Commercially available PCBs are mixtures of PCBs of various degrees of chlorination and the code implies only the average number of Cl-atoms. In Figure 31, 14 different PCBs can be identified in a Chlophen A 50 sample, but many more peaks are obtained if highly sophisticated gas-liquid chromatography is employed. One of the most common ways to quantify chromatograms of PCBs consists in matching the peaks with peaks of known PCB standards. However, this procedure is not very successful since degradation and selective uptake of the PCBs by environmental samples has considerably changed the relative distribution of the PCB peaks so that the environmental sample do not resemble the available standards.

Chau and Sampson (1975) have conducted a comparison of 7 of the most widely used methods in PCB analysis:

1. Peak by peak quantification based on the isomeric distribution and weight-percent of PCB represented in each peak (Webb/McCall method).

2. Mean of all peaks as Aroclor 1254.

3. Mean of all peaks eluated after p,p'-DDE as Aroclor 1254.

4. Total peak height of all peaks as Aroclor 1254.

5. Mean of all peaks as Aroclor 1260.

6. Mean of all peaks eluated after p,p'-DDE as Aroclor 1260.

7. Total peak height of all peaks as Aroclor 1260.

They found that the precision using a certain method was usually sufficient but that the accuracy in measuring the PCB content of environmental samples appeared to be virtually unknown. PCB analyses of materials which had undergone little change from the time of PCB treatment to analysis, could easily be matched with commercial standards, but not environmental samples which have undergone photo-alterations or discrimination of the various PCBs during accumulation.

The Webb/McCall method proved to be the best method, i.e. the one with a minimum spread of values. Reasonable results were also obtained with methods 2 and 5. As expected, no single peak or group of peaks could be selected as representative of the total PCB content.

In addition to the seven methods mentioned above, the quantification based on a single peak after total chlorination of the PCBs present in the sample is worth mentioning. In this methodology all PCB components are perchlorinated to decachloro-biphenyl (DCB) with antimony pentachloride (Armour, 1973). Although this method will not allow the identification of the single components of the PCB, it has the advantage of supplying a single peak, thus increasing the sensitivity and facilitating quantification of the total PCB content. It might further be used to check the total PCB concentration obtained from the other quantification techniques discussed above. Recently, Trotter and Young (1975) have observed that different batches of $SbCl_5$ are contaminated to various degrees with DCB and also bromononachlor-biphenyl, which requires careful checking of the contaminated level of the $SbCl_5$ used. If nonchlorinated biphenyls are present in the sample, they will, of course, also be perchlorinated by this treatment.

Identification by chemical transformation

Since several substances can have the same retention time, a confirmation of the identity is necessary. Rerunning the sample with different GLC columns (ICES, 1974) and comparing retention times before and after a chemical transformation allows the identification of certain substances on the chromatogram. For example, a treatment with an alcoholic NaOH or KOH solution will dehydrochlorinate DDT and DDD and transform them into DDE and DDMU respectively. At the same time, the lipids are hydrolysed without changing the PCBs, so the method can also be used as a clean-up for a PCB analysis.

Chau and Lanouette (1972) proposed a solid matrix for the alkaline transformation confirmation of DDT, DDD and several other pesticides. Miles (1972) reports on an easy dehydrochlorination with 1.5 diazobicyclo (5.4 D) undec-5-ene (DBU) and a successive oxidation by chromic acid to dichlorobenzophenones as a means for both confirmation of the DDTs and their separation from the PCBs. Treatment with K-dichromate in sulphuric acid is reported to remove DDE without altering the PCBs (Westöö and Norén, 1970). DDE can also be oxidized with chromic trioxide in acetic acid to dichloro-benzo-phenone (Mulhern et al., 1971). The previously mentioned conversion of all PCBs by total chlorination to DCB can also be used as a confirmation test (Armour, 1973).

6.6 Gas Chromatographic Analysis of Methyl Mercury

Gas chromatography is usually employed to determine methyl mercury (Hg_M). An alternative method, not yet sufficiently tested utilizing AAS is described under section 6.4.1.

The procedure of GLC analysis takes the following steps:

1. Homogenize the sample (0.5-5.0 FW).
2. Liberate the Hg_M from its protein bond with a strong acid (2.3N, HCl, Hbr, alkali bromide salt in H_2SO_4, NaBr in HCl).
3. Extract the Hg_M with an organic solvent (benzene or toluene).
4. Separate Hg_M from interfering impurities by extraction with an aqueous or ethanolic solution containing a thiol compound (cysteine, glutathione, thiosulphate).
5. Liberate Hg_M from the thiol compound with an acid as in step 2.
6. Extract again with an organic solvent.
7. Analyse in gas-liquid chromatograph.

Detection limit is 1 µg/kg FW with a standard deviation of 2% at concentrations above 50 µg Hg_M/kg FW.

Recovery is about 95% of Hg.

In a recent review prepared as a working paper for the FAO/WHO Expert Consultation to Identify the Food Conaminants to be Monitored and to Recommend Sampling Plans and Methodology, Westöö (1974) recommended the following Hg_M method as reference method (Fig. 33). Jensen et al. (1975) describe a similar procedure.

The following description of the analytical procedure proposed by Westöö (1974) has been reproduced by kind permission of the author:

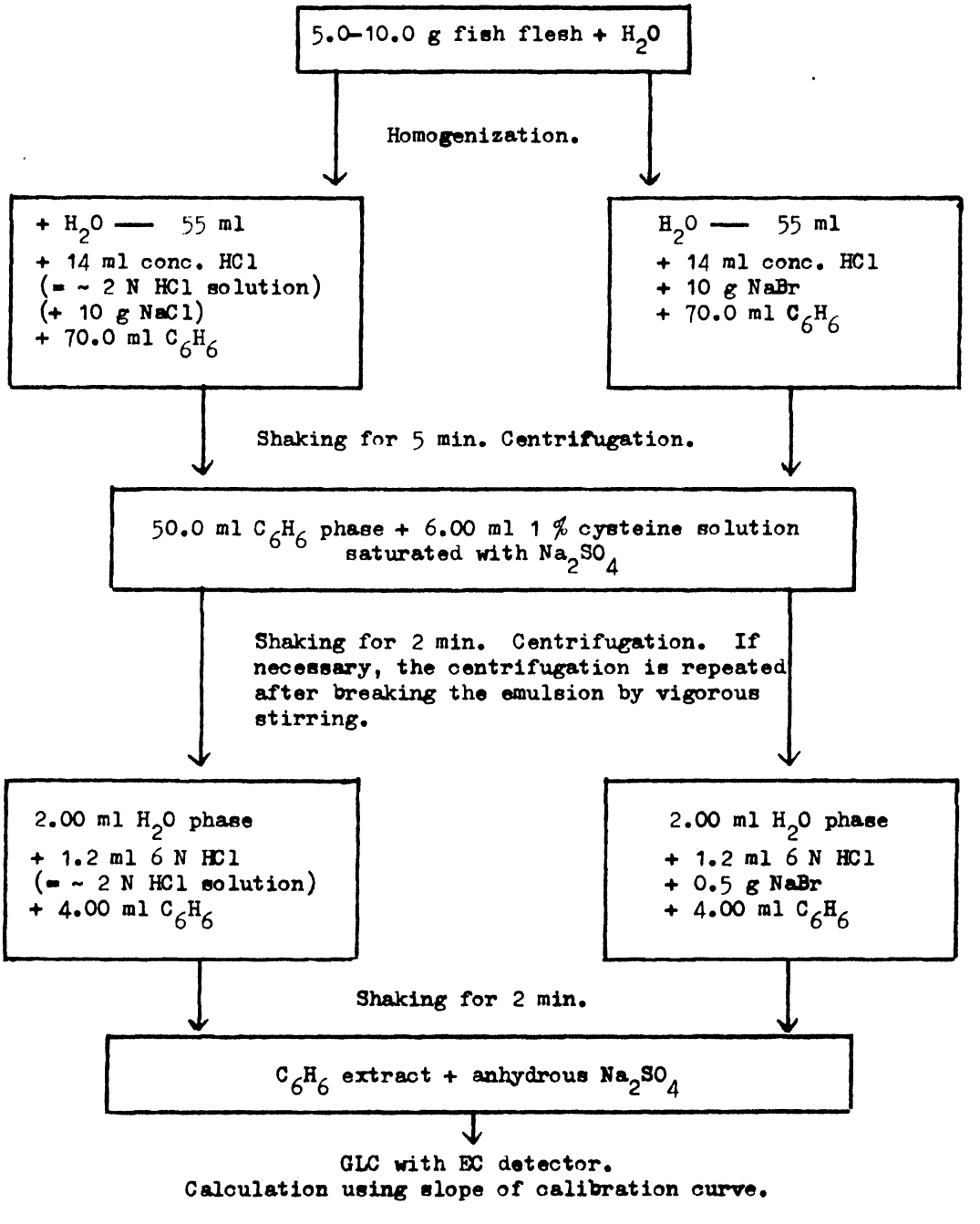

Fig. 33 Analysis of methylmercury in fish flesh (Westöö, 1974)

ANALYSIS OF METHYL MERCURY IN FISH FLESH

Apparatus

(a) Adapter with two ground joints 29/32 and one ground joint 14/23 with thermometer

(b) Centrifuge

(c) Centrifuge flasks, 200 ml (cork stoppers covered with viscose or polyethene film)

(d) Centrifuge tubes, 10-15 ml

(e) Coil condenser with ground joint 29/32, length of coil 20 cm

(f) Distillation column, Widmer, with two ground joints 29/32, effective length 20 cm

(g) Erlenmeyer flasks, 200 ml and 2 l

(h) Flask, round bottom, 3 l, with ground joint 29/32

(i) Gas chromatograph with electron capture detection (^3H) and 1 mV recorder. Column: 5' x 1/8" glass column with lithium chloride and 5% phenyl diethanolamine succinate (PDEAS) on Chromosorb W, AW, DMCS, 60/80 mesh. Gas flow rate: 60-75 ml N_2/min, purified by Molecular Sieve 13 A, 30/60 (Varian). Column temperature: 175°C. Injection temperature: 200°C. Detector temperature: 205°C. Retention time of methylmercury peak: 1.5 min

(k) Graduated cylinder, 100 ml

(l) Heating mantle for 3 l flask, 550 W, with energy regulator

(m) Homogenizer, e.g. Polytron

(n) Pipettes: 1.00, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00, 15.00 and 50.0 ml bulb pipettes and 5.0 ml graduated pipette

(o) Separating funnels, 75 ml

(p) Shaking machine

(q) Test tubes with ground joints, 5 and 15 ml

(r) Vogel pipettes, 70.0 ml, calibrated, and 14 ml

(s) Volumetric flasks: 50.0, 100.0, 200.0, 500, and 1 000 ml

All glassware should be cleaned first by ordinary laboratory washing, then by rinsing with 1N ammonium hydroxide solution deionized water and ethanol.

Filling of column for gas chromatograph, conditioning, etc: Dissolve 0.5 lithium chloride and 1.5 phenyl diethanolamine succinate in 2.5 ml ethanol and 50 ml acetone in a round bottom flask. Add 10 g Chromosorb S, 60/80 mesh, AW, DMCS. Evacuate the flask until all air bubbles have disappeared. After 10 minutes, transfer the mixture to a glass filter funnel and remove the liquid by suction. Air-dry on a filter paper.

Fill the column with the dry Chromosorb coated with PDEAS and LiCl according to prescriptions in handbooks.

Heat the column under low gas flow rate (25.30 ml N_2/min) at 210°C for 18 h (detector end of the column not attached).

Adjust the apparatus to normal conditions (see i). Saturate the column with methyl mercury by injecting large amounts of methyl mercury standard solution repeatedly (about 10 ng each time). If the preparation of the column has succeeded, an injection of 0.10 ng Hg as methyl mercury should give a peak height of at least 20% of the total deflection. If the sensitivity is not good enough and if the peaks are broadened, the column will improve by being kept at working temperature for some days, benzene solutions of methyl mercury iodide or methoxyethylmercury iodide being injected repeatedly during this time (about 40 ng Hg each injection). These solutions are prepared by adding sodium iodide to water solutions of methyl mercury chloride or methoxymethylmercury hydroxide and extracting with benzene (dry the benzene layer with sodium sulphate).

Reagents

Chemicals should, if possible, be of reagent grade and the water should be distilled and deionized.

1. Benzene. Distil with Widmer column (a, e, f, g, h, l).

2. Cysteine solution. Dissolve 1.00 g cysteine hydrochloride (1 H_2O), 0.775 g sodium acetate (3 H_2O) and 12.5 g anhydrous sodium sulphate in water and dilute to 100 ml.

3. Hydrochloric acid, concentrated.

4. Hydrochloric acid, 6 N. Mix equal volumes of water and concentrated hydrochloric acid.

5. Sodium bromide.

6. Sodium sulphate, anhydrous. Dried overnight at 450°C.

7. Standard solutions of methyl mercury:

   (a) Stock solution. Prepare a stock solution containing 100 µg Hg/ml as methyl mercury by dissolving 0.1254 g methyl mercury chloride in water and diluting to 1000 ml. Is stable for years.

   (b) Calibration solution. For calibrations dilute 2.00 ml of the stock solution to 200.0 ml with water. The solution, which contains 1.00 µg Hg/ml, should not be older than 2 days.

   (c) Standard solutions for the gas chromatograph. Dissolve 0.125 g methyl mercury chloride in 1000 ml benzene. Dilute 5.00 ml to 500 ml with benzene. Aliquots of this solution are diluted with benzene so that solutions are formed containing 0.005, 0.010, 0.020, 0.030, 0.040, 0.050, 0.100, 0.200, and 0.300 ng Hg/µl (1.00, 2.00, 4.00, 6.00, 8.00, and 10.00 ml are diluted to 200.0 ml and 5.00, 10.00 and 15.00 ml to 50.0 ml). Equivalent amounts of methyl mercury chloride and methyl mercury bromide give the same peak height.

Procedure

Extraction and clean-up

Homogenize 5.00 g fish flesh with water in a 200 ml centrifuge flask or Erlenmeyer flask. Rinse the homogenizer with water in order to collect the fish flesh quantitatively. If an Erlenmeyer flask was used, transfer the homogenate quantitatively into a 200 ml

centrifuge flask. A total of 60 ml water should be used for the homogenization and transfer. Add to the homogenate 14 ml conc. hydrochloric acid and 10 g sodium bromide. Mix. Add 70.0 ml benzene and shake the mixture for 15 minutes in a shaking machine or for 5 minutes by hand. Centrifuge. Transfer 50.0 ml of the benzene extract to a separating funnel. Add 6.00 ml cysteine solution with a pipette and shake vigorously for 2 minutes. Centrifuge the water layer (often containing emulsion). Discard the benzene phase. If much emulsion is still present, break it by vigorous stirring with a glass rod and centrifuge again. Transfer 2.00 ml of the clear water layer into a 15 ml test tube with ground joint. Acidify with 1.2 ml 6 N hydrochloric acid, add 0.5 g sodium bromide and extract with 4.00 ml benzene by shaking for 2 minutes. Transfer the extract into a 5 ml test tube with ground joint. Dry with anhydrous sodium sulphate. Analyse by gas chromatography. Use a calibration curve for the calculation.

When the methyl mercury level in the sample is expected to be so high that, on injection into the gas chromatograph of the purified extract, the peak obtained will be beyond the linear part of the standard curve, either less sample must be weighed in or the purified extract must be diluted to proper concentration. If low methyl mercury levels are expected, the sensitivity of the method can be increased by shaking the benzene extract with a smaller volume of cysteine solution than normally and changing the subsequent volumes in proportion, or by increasing the amount of sample analysed.

If the analysis has to be interrupted, this should be done after the first centrifugation or before the gas chromatography.

Gas chromatography

Immediately before and after the injection of the sample, inject a standard with a concentration differing only slightly from that of the sample, so as to obtain about the same peak height of sample and standard without the volumes of sample and standard differing more than 25%. The error, which can arise from calculating the methyl mercury concentration of the extract, as if the standard curve based on peak heights always went through the origin, will hereby be insignificant.

Usually, the extract are stable and can be stored for several weeks, but at a few occasions when the extracts had been kept for a couple of months, a loss of methyl mercury was observed.

Standard curve

Inject into the gas chromatograph 5.0 and 7.0 µl of standard solutions containing 0.050, 0.100, 0.200 and 0.300 ng Hg/µl. Plot in a diagram the peak height against amount of mercury injected. A straight line cutting the x-axis at most 0.05 ng Hg from the origin should be obtained.

Calculation of Results

In this procedure only a few per cent of the methyl mercury is lost by incomplete extraction. In spite of that a calibration curve should be made every week, because this is a simple way of controlling that the standard solutions have not changed by evaporating and that the GLC system gives correct results.

Calibration curve

Analyse 1.00, 2.00, 3.00, 4.00 and 5.00 ml of the methyl mercury chloride standard solution (1.00 µg Hg/ml) according to the above procedure, only exchanging the fish sample for v ml standard solution. Centrifuging is not needed. Inject the purified extracts into the gas chromatograph. Plot 200 x added mercury in mg along the y-axis in a rectangular coordinate system. A straight line through the origin is obtained, when the points are combined. By multiplying the mercury concentration of the purified fish extracts in ng/µl

with the slope of this line the methyl mercury level of the fish expressed in mg Kg/kg is obtained. (When other amounts of the samples than 5 g are used, when the extracts are concentrated by the application of a smaller volume of cysteine solution than normally or when the extracts are diluted, the levels must be corrected.)

Blank

Exchange the fish sample for 5 ml water. Proceed in all other respects according to the above description. The blank should give no peak at the retention time of methyl mercury.

7. STATISTICAL CONSIDERATIONS

7.1 General considerations

Many texts on statistics of different degree of mathematical sophistication are available in English and other languages. It is, therefore, not necessary to repeat concepts and procedures already well described elsewhere. However, a few topics, especially relevant to the pilot projects, are worthwhile discussing. These include: statistical implication of composite or pooled samples, precision of analytic methods and biological variability, strategies for the selection of samples for analysis and sampling plans.

These topics will be discussed in reference to three elementary texts:

1) 'Statistical Methods' in Documenta Geigy, (Diem, 1962) (in English with French, Spanish and German versions).

2) 'Statistical Methods' by Snedecor and Cochran (1967).

3) 'Experimental Statistics' by Gibbons Natrella (1963).

Snedecor and Cochran's, and Gibbons Natrella's texts give many detailed examples of application. The symbols used here are those of Diem (1962), since this text is translated into several languages.

In the following text, some confusion may arise from the different use of the word 'sample'. In the section on sample collection and sample preparation the word 'sample' implies an aliquot or specimen collected. In statistics a sample taken from a statistical population of data consists of n values of the random variable x_i. In order to avoid confusion between the two meanings in this paragraph, the word sample will be written between quotation marks (" ") if it is used in the meaning of "sample" collection and preparation.

7.2 Mean, median and composite "sample"

Mean : The mean of n determinations (x_i) of the amount of a pollutant is

$$\bar{x} = \frac{x_1 + x_2 + \ldots + x_n}{n} = \frac{\sum_i x_i}{n} \qquad (I)$$

The mean concentration $\bar{c}$ of a mixture of n "sample" of the weight g_i with the concentration c_i is

$$\bar{c} = \frac{g_1 c_1 + g_2 c_2 + \ldots + g_n c_n}{g_1 + g_2 + \ldots + g_n} = \frac{\sum_i g_i c_i}{g_T} \qquad (II)$$

where g_T is the total weight of all "samples".

"Composite sample" :

The mean value can also be determined by analysing a "composite sample" obtained by mixing homogeneously n "samples" of the weights g_i and the concentrations c_i. Since in a "composite sample" the single concentrations c_i are not determined, and therefore unknown, only when all weights are equal (i.e. $g_1 = g_2 = \ldots = g_n$) is the mean concentration $\bar{c}$ equal

to the sum of the single concentrations of the individual "samples". The determination of a concentration in a "composite sample" is a very efficient method for the estimation of the mean of a set of "samples", since this estimation is based on one single determination. The only necessary requirement consists in mixing "composite samples", the aliquots taken from the different specimens, should be approximately the same weight or volume. For example this can be achieved during "sample" preparation by treating the smallest organisms first and reducing the weights of all larger "samples" to the weight of the first "sample".

Median :

In non-normal distributions the median is often preferred to the mean. If the total number n is an odd number, the median $\tilde{x}$ is the middle value of a set of n data x_i ranked in order of magnitude. The median is the arithmetical mean of the two middle values if n is an even number.

Variance :

The variance supplies an estimation of variability for both normal and non-normal distributed data. Most statistical tests assume that the data are normal or near normal distributed. It is, therefore, necessary to test for normality before applying these tests.

The variance s^2 of a sample of n determinations x_i is :

$$s^2 = \frac{\sum_i (x_i - \bar{x})^2}{n-1} = \frac{\sum_i x_i^2 - \frac{(\sum_i x)^2}{n}}{n-1} \qquad \text{(III)}$$

It is an unbiased estimation of the population variance σ^2.

The variance of the sample mean $\bar{x}$ is :

$$s_{\bar{x}}^2 = \frac{s^2}{n} \qquad \text{(IV)}$$

The variance can also be estimated from duplicate determinations of different "samples" of the same matrix. If $d = x_2 - x_1$ is the difference between duplicate determinations of the same "sample" then

$$s^2 = \frac{\sum_i d_i}{2p} \qquad \text{(V)}$$

where p is the number of duplicate pairs and $2p = n$ is the total number of all determinations.

The variance s^2 of k independent variances s_i^2 is :

$$s^2 = \frac{(n_1 - 1)\, s_1^2 + (n_2 - 1)s_2^2 + \ldots + (n_k - 1)s_k^2}{n - k} \qquad \text{(VI)}$$

A quick way of estimating the standard deviation s is given by the range d of a sample:

$$s = d \cdot f_n \qquad \text{(VII)}$$

where f_n is a factor which depends on the number n of "samples" which make up the sample. For $n = 2$, $f_2 = 0.886$, $f_3 = 0.591$ and $f_4 = 0.486$. The efficiency of estimating s diminishes rapidly when more than two values are available, (III) is then much more efficient.

7.3 Confidence limits

The estimation of the population mean μ, and standard deviation σ from the corresponding sample parameters does not give any information about the probability of deviations from the population parameters. Calculating the confidence limits from a sample will supply an interval which for a given probability will enclose the parameter. The confidence limits allow an easy and fast inference about the significant difference between parameters.

Mean : The 95% confidence limits of a sample mean are estimated by :

$$\bar{x} \pm \frac{t_{n-1} \cdot s}{\sqrt{n}} \qquad \text{(VIII)}$$

assuming a normal or near-normal distribution. Values for t can be obtained from the Student distribution in tables supplied in most statistical texts.

Standard deviation :

From a sample estimation (s) the confidence limits for the true standard deviation σ are:

$$L_l = k_l s_\nu \; ; \; k_l = \sqrt{\frac{\nu}{\chi^2_{\nu_1\, 1-\alpha}}}$$

$$L_r = k_r s_\nu \; ; \; k_r = \sqrt{\frac{\nu}{\chi^2_{\nu_1\, 1-\alpha}}} \qquad \text{(IX)}$$

where ν is the degree of freedom (DF), χ^2 = chi square distribution with ν DF and the probability α. L_l and L_r are the lower and upper confidence limits, the chi-square distribution is tabulated in most texts, often also the values for k_l and k_r are given.

7.4 Sources of variance

In the monitoring program four different sources of variation can be distinguished:

1) Variability of the chemical analytical method ('between determinations').

2) Variability of the chemical pretreatment ('between pretreatments').

3) Variability caused by intrinsic (genetic) biological factors ('between "samples" same site and same date').

4) Variability caused by environmental factors ('between sites and date' or 'between dates, same site').

Knowledge about these variabilities will greatly increase the efficiency and the precision of the monitoring program, and supply at the same time, the information necessary for establishing differences between pollutant concentrations and trends in the degree of contamination.

Variance of the chemical analytical method (instrument variance)

In setting up the chemical method this variance is usually estimated from repeated determinations of a given standard and calculated according to (III). The variance can also be estimated from (V) during the determination of pollutant concentration in environmental "samples". It requires only duplicate determinations of the same pretreated "sample" which in many analytical procedures are not time consuming and are easy to carry out. Determining the chemical analytical variance on actual "samples", instead of standard solutions, has the advantage that possible matrix effects are included in the estimation of the variance. If duplicated determinations are costly they may be carried out occasionally to check if the variance remains constant during the course of the investigation. The result of the estimation is usually given as standard deviation from the mean s derived from IV.

Variance of the chemical pretreatment

This variance can be estimated by running the same "sample" several times through the pretreatment procedure. The variance is then estimated with (III). An alternative method consists in carring out the pretreatment in duplicates of different "samples" of the same matrix and estimate the variance with (V) and the standard deviation of the mean with (IV).

The variance of the entire chemical analysis i.e. the variance of the instrument and of the pretreatment is then the sum of both variances (V) and their standard deviation is then obtained from (VI) where $n = n_1 + n_2 + \ldots + n_k$. This variance and standard deviation estimated the precision of the chemical method.

Variance of the intrinsic biological factors

The intrinsic biological variance is estimated from the determination of pollutant concentrations in specimens which were collected in homogeneous biotopes where important environmental factors vary little. Analysing a certain number of specimens from the same depth, salinity, temperature, similar exposure to contamination, etc. will allow the estimation of this variance. For certain pollutants (accumulative pollutants) such as Hg, DDT and others, a correlation between age and concentration has been observed (Cumont et al., 1972; Cross et al., 1973). This correlation influences the variance estimations.

Variability caused by environmental factors

The estimation of this variance is probably the most important, since it will probably be the greatest of all the variances encountered. For example, seasonal fluctuations of metal concentration in coastal regions have been observed (e.g. Ireland, 1974). Increasing pollutant concentration in the water towards the pollution source coincided with increasing concentration in the marine invertebrates studied (Butterworth et al., 1972; Nickless et al., 1972; Peden et al., 1973). Fluctuation in the exposure to pollutants (e.g. changes in trace concentrations inputs from rivers) will reflect the pollutant concentration in the organisms, but other factors will also intervene. Greater availability of food organisms increases the accumulation of heavy metals (Nassogne, 1972). Since for most pollutants the greatest enrichment step occurs from the sea water to first trophic level (phytoplankton and macrophytes) fluctuations in primary production during the seasons can result in fluctuations in pollutant concentrations. Analysing different "samples" will allow the estimation of this variance. Establishing correlations with environmental factors will improve the efficiency of the estimation.

7.5 A strategy for the selection of "samples" for analysis

Knowledge about the possible sources of variance and their relative magnitude allows the establishment of priorities in the determination of the different variances. Since the variability caused by the environmental factors is likely to be the greatest and also the one which will vary the most between sites and dates, all additional effort after the analysis of a "composite sample" should be directed to determine this variance in each site.

Once the analytical and pretreatment variances are estimated, they need only be checked occasionally to verify that the precision of the chemical procedure is still the same. In AAS, for example, the analytical precision is easily checked and without much effore by running duplicates of the samples through the AAS. In GLC analysis duplicates require considerably more time and it is probably more efficient to check the precision of the entire analytical chemical method including pretreatment since the evaluation of the GLC output is time consuming. A scheme of this strategy is shown in Table V.

For the sampling programme this means that in addition to "composite samples" for each site in each season a limited number of single "specimen samples" and some additonal "composite samples" have to be collected and prepared.

The "sample" preparation of Mullus barbatus can, therefore, be modified by using one fillet for the "composite sample" and the other as a "specimen sample" which is stored (deep frozen) separately. "Composite samples" prepared from different specimens collected in the same site at the same date will supply duplicates of the "site composite sample".

Crustaceans and mussels do not posses two easily separated parts. Either the soft parts are divided in two or different specimen are used to prepare the "composite sample" and the single "specimen samples". Using different specimens is especially recommended for mussel "samples", where probably even one mussel might not give enough material for chemical analysis during certain seasons.

7.6 Criteria for a quick comparison of means and standard deviation

In statistical texts many different tests are described which can be used to establish whether means are significantly different from each other. Here only the criteria based on confidence limits which allow a fast and easy interference are discussed. The use of confidence limits has two advantages over statistical tests (Sachs, 1970). The confidence limits use the same scale as the data and they give an immediate idea of the precision of the mean.

The following criteria can be used to decide if two parameter are different at the significance level of the confidence limits:

1) If the L_r of the smaller parameter is smaller than the L_1 of the larger parameter then there exists, at the chosen significance level, a true difference between the two parameters. Or in other words, if the confidence intervals of the two parameters do not overlap, the parameters are different.

2) If L_r of the smaller parameter is greater than the L_1 of the greater parameter, but the wider confidence interval of the two parameters do not cover more than $\frac{1}{4}$ of the narrower confidence interval, a statistical test may be used to establish a significant difference.

Table V A strategy for analysing "samples" with AAS and GLC

	initial effort	effort during AAS	monitoring GLC
Analytical method	5 - 10 replicates during setting up methodology or duplicates when estimating variance of pretreatment	duplicates together with standards	none, checked together with pretreatment
Pretreatment	5 - 10 replicates of pretreatments during setting-up of the methodology or duplicates of sample pretreatment	duplicate pretreatments with standards or when changing matrix	as in AAS
"Specimen sample" ('same-site-same-date')	duplicate "samples" at the beginning of the programme in a given site	duplicate "sample" when possible, at least once a year	as in AAS
"Site composite samples" ('same-site-different-date' or 'same-date-different-site')	replicates of "composite samples"	"composite samples" whenever possible in duplicates	as in AAS

Table VI Hierarchic order of sources of variance and the estimation of the means and variances

	variable	mean	variance[1/]
1. Chemical analytical method	x_c	$\bar{x}_c = \frac{\sum x_c}{n_c}$	$s_c^2 = \frac{\sum (x_c - \bar{x}_c)^2}{n_c - 1}$
2. Pretreatment	$x_p = \bar{x}_c$	$\bar{x}_p = \frac{\sum x_p}{n_p}$	$s_p^2 = \frac{\sum (x_p - \bar{x}_p)^2}{n_p - 1}$
3. "samples" from specimens	$x_s = \bar{x}_p$	$\bar{x}_s = \frac{\sum x_s}{n_s}$	$s_s^2 = \frac{\sum (x_s - \bar{x}_s)^2}{n_s - 1}$
4. sites ("composite samples")	$x_{si} = \bar{x}_s$	$x_{si} = \frac{\sum x_{si}}{n_{si}}$	$s_{si}^2 = \frac{\sum (x_{si} - \bar{x}_{si})^2}{n_{si} - 1}$

1/ The variance can, of course, also be estimated from equations (VI)

Note that $\bar{x}_s$ is equal to the value obtained from analysing a "composite sample"

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Appendix A

Preparing plastic tweezers

Methylmetacrylate of 4 mm thickness has been found to be very useful as it will give the right elasticity. If thinner or thicker material has to be used, either the strips from which the tweezers are to be made are cut wider or narrower. The easiest way to heat the plastic and bend it is with a hot air blower used for forming plastics. A drying oven brought to 135-140°C can be used, however, it is much more difficult to make tweezers by heating the plastic in an oven since the plastic twists easily.

Material :

- Sheets of acrylic (methylmetacrylate) resin; 4 mm thick (Trade names: e.g. Perspex, Plexiglas, Lucite)
- A plastic tube of 40 mm diameter

Equipment :

- A hot air blower (300-350°C) for plastics (e.g. Karl Leister, Switzerland, W 1850, 400 rpm)
- Or a drying oven at 135-140°C
- A plastic tube of 40 mm diameter

Procedure : with a hot air blower

1. Cut with an electric or a hand saw strips of about 10 mm width and 250 mm length.
2. Heat about a 60 mm long part in the middle of the strip so that it will bend easily and bend it around the plastic tube carefully in order to make both ends meet. Cool the plastic with cold water.
3. Sharpen the end with a file and roughen the inside of the tweezers so that they grip well.
4. Wash the tweezers carefully with detergents and rinse them with distilled water.

: with a drying oven

1. Place the plastic strips on a clean piece of wood in a drying oven (135-140°C) until the plastic becomes soft.
2. Lift the plastic at one end with a pair of tweezers from the oven letting the other end hang down so that the plastic will not bend.
3. Bend it around the plastic tube without letting the tweezer tips meet and cool the tips immediately by dipping them in a beaker of clean cold water so that the tips of the tweezers do not curve.
4. Now bend the tips of the tweezer so that they will meet.
5. Prepare the points as described earlier.

Appendix B

Recommended wavelengths, optimal working ranges and typical sensitivities (Parker,1972) *

Element	Wavelength nm	Optimal range ng/ml		Typical sensitivity ng/ml	Spectral band pass nm
As	193.7	50000 -	200000	780	1.0
	197.2	60000 -	250000	1200	1.0
Cd	228.8	500 -	2000	11	0.5
	326.1	250000 -	1000000	4600	1.0
Cr	357.9	2000 -	8000	55	0.2
	425.4	10000 -	40000	170	0.2
	428.9	25000 -	100000	410	0.2
	520.8	650000 -	2600000	11000	0.1
	520.4	1500000 -	6000000	28000	0.1
Cu	324.7	2000 -	8000	40	0.2
	327.4	6000 -	24000	140	0.2
	217.9	15000 -	60000	330	0.2
	218.2	10000 -	80000	440	0.2
	222.6	70000 -	280000	1500	0.1
	249.2	200000 -	800000	4900	0.5
	244.2	500000 -	2000000	11200	1.0
Hg	253.7	100000 -	400000	2200	0.2
Mn	279.5	1000 -	4000	24	0.2
	403.1	15000 -	60000	300	0.2
	321.7	3500000 -	14000000	65000	0.1
Ni	232.0	3000 -	12000	66	0.2
	341.5	15000 -	60000	340	0.2
	352.4	15000 -	60000	340	0.2
	351.5	35000 -	140000	720	0.2
	362.5	2000000 -	8000000	36000	0.2
Pb	217.0	5000 -	20000	110	1.0
	383.3	10000 -	40000	230	0.2
	261.4	200000 -	800000	4000	0.2
	202.2	250000 -	1000000	5600	0.2
	205.3	2000000 -	8000000	38000	0.2
Se	196.0	20000 -	80000	480	1.0
	204.0	300000 -	1200000	6800	0.3
Zn	213.9	400 -	1600	9	0.2
	307.6	3500 -	14000	76000	0.5
Ca	422.7	1000 -	4000	21	0.2
	239.9	200000 -	800000	4200	0.1
K	766.5	500 -	2000	10	0.5
	769.9	1500 -	6000	30	0.5
	404.4	200000 -	800000	3700	0.1
Mg	285.2	100 -	400	3	0.5
	202.5	5000 -	20000	90	1.0
Na	589.0	150 -	600	3	0.3
	589.5	500 -	2000	8	0.3
	330.2 / 330.3	100000 -	400000	1600	0.3

* C.R. Parker (1972), Water analysis by atomic absorption spectroscopy. Springvale, Vic. Australia, Varian Techtron, 78 p.

Appendix C

Homogenizing of samples

The use of homogenized samples has many advantages, but during the homogenization the samples can very easily be contaminated. Homogenization of entire organisms is difficult to achieve since an uniform distribution of all different tissues in the sample is practically impossible to obtain. Especially the more resistant tissues, such as fish skin and bones or the internal shell of sepia, cannot be completely amalgamated with the other tissues so that identical subsamples can be drawn. Therefore, homogenizing should be limited to already homogeneous tissues (fish fillets, soft parts of molluscs, muscle tissue of crustaceans, etc.).

Homogenizing the same tissues of several specimens (composite sample) will be necessary to provide material for a sufficient number of identical subsamples, especially of smaller organisms for several analyses and at the same time allows a very efficient determination of the mean value of the concentration of an analyte in several specimen samples with theoretically only one determination (see section 7.2).

The greatest disadvantage of homogenizing tissues of single specimen samples and preparing composite samples of different specimens lies in the contamination hazard arising from the use of the homogenizer.

Homogenizing for chlorinated hydrocarbon analysis

The contamination hazard of homogenizing samples for Chl-HC analysis are easier to avoid than those for metal analysis, since homogenizers entirely made from stainless steel are commercially available (e.g. Warren blender, Turrax homogenizer, etc.). The Warren blender or similar apparatus are used in the extraction procedure of the Chl-HC analysis (see section 6.5.2). These apparatus are available with blenders of different volumes, so the same instrument can be used for the homogenization of large samples and the extraction of smaller subsamples.

Contamination hazards arise, especially, from the lubrification oils and the difficulties encountered in cleaning the blender. Not all parts of the blender which come into contact with the sample can be cleaned by heat decontamination at 450°C. Therefore accurate cleaning with detergents followed by thorough rinsing with solvents is necessary. Blank check runs with the concentrated solvent used for cleaning are recommended.

Homogenizers using vibrating cylinders to which solvent extracted clean quartz sand (baked for 24 hours at 450°C) is added together with the sample may provide an alternative to the blenders. If available, mills used to fractionate geological samples utilizing cylinders which rotate eccentrically at high speed, constructed of stainless steel or metal carbids, could also be used. These mills are, however, considerably more expensive than the blenders mentioned above.

Homogenizing for metal analysis

Avoiding metal contamination while homogenizing biological samples for metal analysis is considerably more difficult and the apparatus or the procedure to be employed depend on the element to be analysed.

For elements which are not contained in stainless steel as constituents but only as possible impurities (e.g. Hg, As, Se, etc.) stainless steel blenders and homogenizers as those used in Chl-HC analysis can be employed. However, only blank trial runs can ascertain whether a certain blender can be used. Since it is not always easy to foresee which element samples will be analysed in the future, it is advisable to homogenize the sample with a stainless steel apparatus only immediately before the actual analysis in order to allow the analyst to select the most suitable homogenizer for each case. This implies that composite samples are deep frozen and stored after sample preparation (see section 5) without being homogenized immediately.

A very simple, but somewhat laborious way of preparing composite samples, especially from fish fillets and shrimp tail muscles, is to cut the samples with the help of a clean plastic knife and plastic tweezers on a clean glass surface (e.g. Pyrex dish) into small, approximately even sized pieces after sample preparation (see section 5) and to mix the pieces thoroughly in a clean plastic container so that the pieces are randomly distributed in the sample. Subsamples of appropriate size can then be prepared for deep freezer storage.

For very soft tissues homogenizers made from Teflon and Pyrex glass can be considered. An alternative method is to homogenize deep frozen samples in an agata mortar with a pestle. Clean quartz sand may be added during the homogenization. However, this is also quite a laborious technique. Digestion of composite samples in large Teflon containers and storage of the resulting solution cannot be recommended since the strong acid digest will attack the container surface and thus contaminate the sample.