REGIONAL SEAS

UNITED NATIONS ENVIRONMENT PROGRAMME

12 November 1984

Sampling of selected marine organisms and sample preparation for trace metal analysis

Reference Methods for Marine Pollution Studies No. 7 Rev. 2

Prepared in co-operation with

FAO IAEA IOC

UNEP 1984

Note: This document has been prepared in co-operation between the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Intergovernmental Oceanographic Commission (IOC) of UNESCO and the United Nations Environment Programme (UNEP) under projects FP/ME/0503-75-07, ME/5102-81-01, FP/5102-77-03 and FP/5101-84-01.

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes ten regions and has over 120 coastal States participating in it. 1/ 2/

One of the basic components of the action plans sponsored by UNEP in the framework of Regional Seas Programme is the assessment of the state of marine environment and of its resources, of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of reference methods and guidelines for marine pollution studies are being developed and are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations system as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

International Laboratory of Marine
Radioactivity
International Atomic Energy Agency
c/o Musée Océanographique
MC98000 MONACO

which is responsible for the technical co-ordination of the development, testing and intercalibration of reference methods.

1/ UNEP: Achievements and planned development of UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1 UNEP, 1982.

2/ P. HULM: A Strategy for the Seas. The Regional Seas Programme: Past and Future UNEP, 1983.

This issue (Rev.2) of the Reference Method for Marine Pollution Studies No. 7 was prepared in co-operation with the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and the Intergovernmental Oceanographic Commission (IOC) of UNESCO. It includes comments received from IOC's GIPME Group of Experts on Methods, Standards and Intercalibration (GEMSI), from the FAO/UNEP/IAEA Experts Consultation Meeting on Reference Methods for the Determination of Chemical Contaminants in Marine Organisms (Rome, 4-8 June 1984) and from a number of scientists who reviewed and tested the method. The assistance of all those who contributed to the preparation of Revision 2 of this reference method is gratefully acknowledged.

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

This publication describes the sampling and sample preparation procedures suitable to obtain uncontaminated samples of mussels (total soft tissue), shrimps (muscles), and fish (muscles) for trace metal analysis by atomic absorption spectrophotometry.

2. REFERENCES

BERNHARD, M. (1976) Manual of methods in aquatic environment research. Part 3 Sampling and analyses of biological material. FAO Fish.Tech.Pap. No. 158 (FIR1/T158), pp. 124. FAU, Rome.

UNEP/FAO/IAEA (in preparation). Guidelines for monitoring chemical contaminants in marine organisms. Reference methods for marine pollution studies No. 6. UNEP, Geneva.

3. PRINCIPLES

Specimens of organisms selected and collected according to UNEP/FAO/IAEA (in preparation) are enclosed in plastic containers and transported to the analytical laboratory either as cooled (-2 to 4°C) or as deep-frozen (-18°C) samples There the specimens are dissected under "clean conditions" and subsamples are prepared for the analyses of trace metals.

4. REAGENTS

4.1 Demineralized distilled water or glass distilled water of equivalent quality, with a trace metal content below detection limits when checked with this reference method.

4.2 Uncontaminated "open-ocean" subsurface (1 m below the surface) sea water.

4.3 Detergent recommended for laboratory use.

5. APPARATUS

5.1 Plastic thermo-insulated boxes (camping equipment) cooled with commercially available cooling bags. For storage and transport of mussels the boxes must be equipped with a grid in the bottom in order to avoid the mussels being submerged when moistened during transport and storage.

5.2 Refrigerator (required for 6.2, 6.3, 6.4).

5.3 Deep-freezer (-18°C).

5.4 Heavy duty, high-density polythylene bags or suitable plastic containers for storage of specimens.

5.5 Plastic length-measuring board, length-measuring scale (ruler) or transparent Pyrex dish (cooking utensil) with centimetre scale attached underneath (for small and medium-size specimens).

5.6 Two or more plastic knives made out of high-density and purity polyethylene or similar material. Alternatively, quartz knives can be used.

5.7 Pyrex dishes or porcelain dishes (cooking utensils) as working surface for sample preparation.

5.8 Two or more pairs of plastic, commercially available or "home-made", tweezers (see Appendix A).

5.9 High density and purity polyethylene bags and airtight plastic containers with screw caps, for preservation of samples in deep-freezer, cleaned with detergent (4.3) and rinsed with distilled water (4.1) or uncontaminated sea water (4.2).

5.10 High-density polyethylene sheets for covering working bench.

5.11 Smaller polyethylene sheets to be used as "weighing plastic".

5.12 Balance (100-200 g) with a precision of 0.001 g or better, for weighing specimens and subsamples; preferably a "top-loading" balance.

5.13 Plastic wash bottle containing glass-distilled water (4.1).

5.14 Scraper (figure 1), a strong rust-free knife or similar for collecting mussels.

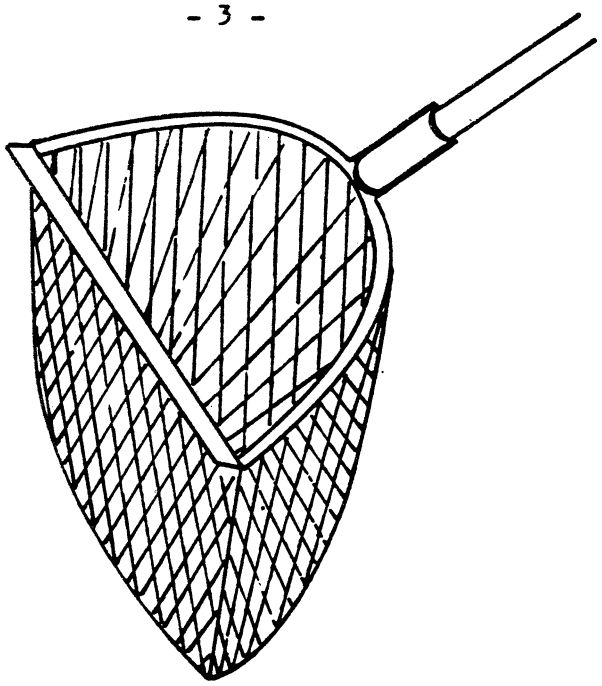

FIGURE 1 : SCRAPER FOR COLLECTING MUSSELS

5.15 Plastic tank or bottle (20 - 50 l) for the sea water (4.2) needed to moisten live mussel samples during storage and transport.

5.16 Large rust-free metal knife for cutting portions from large fishes.

5.17 Stainless steel blender or other tissue homogenizer made from glass and/or teflon. Stainless steel equipment should be tested for trace metal contamination by homogenizing reference (standard) material and comparing the analytical result obtained with same material which was not homogenized with stainless steel equipment.

5.18 Strong plastic brush for removing foreign material attached to the surface of mussels.

6. SAMPLING AND TRANSPORT

6.1 Presampling preparations

Clean the thermo-insulated boxes (5.1), the high density polyethylene bags or containers (5.4), the length-measuring board (5.5), the large rust-free knife (5.16), the scraper or the knife (5.14) with detergent (4.3) and rinse them with distilled water or, alternatively, with clean open-ocean sea water (4.2).

6.2 Sampling of mussels

Remove mussels from their attachments with the clean scraper or the rust-free knife (5.14).

Transfer a suitable number (UNEP/FAO/IAEA (in preparation)) of undamaged mussels into clean thermo-insulated boxes with grid on the bottom (5.1). Collect, from the sampling site, a clean sea water sample in a suitable container (5.15) to keep the mussels moist if a long transport (more than 2 hours in hot climates) is envisaged. Keep the mussels moist with the clean sea water without submerging them.

If the mussels have to be transported and stored before sample preparation (7) for more than 24 hours place a suitable number of mussels in plastic bag (5.9). Squeeze out the air and close the bag airtight with a knot, thermoseal, or similar. Place the bag into another bag (5.4) together with a sample identification note (see Appendix B), close airtight the second bag and deep-freeze.

This represents the "specimen sample".

NOTE: The transport of mussels collected near the laboratory will not present special transport and storage problems. Mussels should be kept exposed to air and moistured with clean sea water during the transport to the laboratory. When gathered from the intertidal zone, they will survive aerial exposure for 24 hours. Mussels submerged in sea water during transport will open their valves, start pumping water and excreting waste products, while during aerial exposure their valves will remain closed and their metabolic rate is greatly reduced; therefore their submersion in sea water during transport should be avoided.

6.3 Sampling of shrimps and small to medium-size fish

Place in a clean plastic bag (5.4) a suitable number of the undamaged specimens (select according to UNEP/FAO/IAEA (in preparation)) collected from a fishing vessel, fish market, etc., taking care that the legs, spines, etc. will not puncture the plastic. Squeeze out the air and close the bag airtight with a knot, thermoseal, or similar. Place the bag into another bag (5.4) together with a sample identification note (see Appendix B), and close the second bag airtight also. Deep-freeze (5.3) the bag whenever possible. Use a refrigerator (5.2) or a cooled thermo-insulated box (5.1) only if the storage period is not too long (48 hours in hot climates).

This represents the "specimen sample".

6.4 Sampling of large-size fish

Determine and note the fork-length, the body weight and sex of the collected specimen.

Separate with a clean rust-free metal knife (5.16) a portion of at least 100 g of muscle tissue. This portion must be at least 5 cm thick so that during sample preparation (7.3) contaminated and dirty tissue can be sliced off. Place each portion into a separate clean bag (5.4), squeeze out the air and close the bag airtight. Place it together with the sample identification note (see Appendix B) into a second bag (5.4) and close it airtight also. Deep-freeze

(5.3) the bag whenever possible, otherwise use a refrigerator (5.2) or a cooled thermo-insulated box (5.1) if the storage period is not too long (48 hours in hot climate).

This represents the "specimen sample".

7. SAMPLE PREPARATION

7.1 Preparatory activities

If necessary, partially thaw deep-frozen samples (6) by placing them overnight in a refrigerator at -2°C to 4°C (partially frozen samples are easier to cut than completely thawed or even fresh samples).

Clean the knives (5.6), the dishes (5.7), the tweezers (5.8), the length-measuring board (5.5) and "weighing plastics" (5.11) with detergent (4.3), rinse with distilled water (4.1) or clean sea water (4.2). Cover the working area with pre-cleaned plastic sheets (5.10). Clean hands carefully with detergent (4.3) and rinse them with distilled water (4.1) or clean sea water (4.2).

NOTE: If hands are cleaned and precautions are taken not to touch the dissected part with hands, bare hands are preferred to hands covered with gloves, since the operator has a much better control of instruments, etc. If possible a clean room should be used for preparatory activities.

7.2 Sample preparation of mussels

Scrape off all foreign materials attached to the outer surface of the shell with a clean plastic knife (knife no. 1) (5.6), to be used only for this purpose or with a strong palstic brush (5.18). Handle the mussels as little as possible.

Rinse each mussel with distilled water (4.1) or alternatively with clean sea water (4.2) and let the water drain off.

Pull out the byssus which extrudes from between the closed shells on the concave side of the shells.

Weigh (5.12) the whole mussel and note the weight.

Insert a second clean plastic knife (knife no. 2) (5.6) into the opening from which the byssus extrudes and cut the adductor muscles by turning the knife as indicated in figure 2 and open the mussel. Do not try to break the mussel open with the knife; if the muscles are cut, the mussel will open easily. Check if the byssus has been eliminated completely; if not, remove the remainder with clean tweezers (5.8).

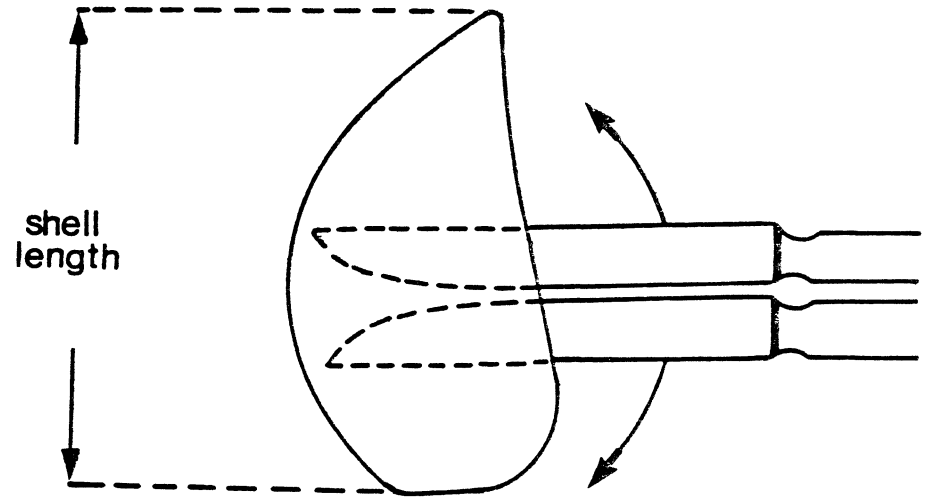

FIGURE 2 : CUTTING THE ADDUCTOR MUSCLE

Rinse the soft part of the mussel in its shells with distilled water (4.1) or clean sea water (4.2).

Loosen all tissue with the second clean knife (knife no. 2) (5.6), remove the soft tissue from the shell with a pair of clean plastic tweezers (5.8) without touching the outer part of the shells, and let all the water drain off.

(a) Single specimen sample: Weigh a clean empty container (5.9) on the balance and note the weight. Then put the soft part of the mussel in it and reweigh. Note the fresh weight of the soft part. Close the container airtight, label it with the sample preparation code. Determine the length of the mussel's shell (figure 2) by placing it with the inner part facing the cm scale (5.13). Note the length of the shell and the weight of the soft part of the mussel.

(b) Composite sample: Fill a container (5.9) of known weight with at least 10 soft parts of mussels prepared as described above. Reweigh the plastic container and note the composite fresh weight of the mussels. Homogenize the specimens in a cleaned blender (5.17), and return the homogenate in the plastic container. Note the total weight again and recalculate the fresh weight of the homogenate. Lable the plastic container with the sample code.

NOTE: When preparing composite samples, use mussels of similar size. The length and weight of each specimen should be determined separately before the soft parts are pooled.

Place several plastic containers in a clean plastic bag (5.4), include an identification note with the containers sample codes, seal the bag airtight and deep-freeze.

This represents the "tissue sample".

7.3 Sample preparation of shrimps

(a) Single specimen sample: Determine the length of the shrimp from rostrum to uropod (see figure 3) using the appropriate length measuring device (5.5). Weigh the shrimp after placing a clean "weighing plastic" (5.11) on the balance (5.12) and note its length and fresh weight.

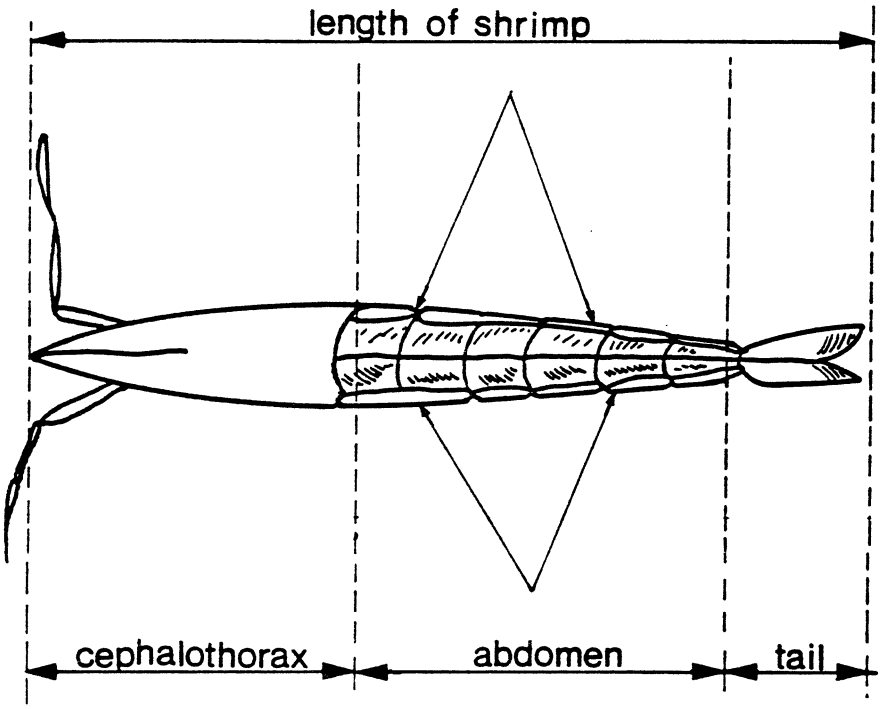

FIGURE 3 : SCHEMATIC DIAGRAM OF A SHRIMP
(arrows indicate where to cut after the legs have been removed)

Separate the abdomen from the cephalothorax and the "tail" (telson and uropod) with a first plastic knife (knife no. 1) taking care that no viscera remain in the abdomen (figure 3). Cut off all legs. Turn the abdomen with the ventral side up and cut with a plastic knife along the edges of the sterinites (ventral exoskeleton); lift the sterinites off with a pair of plastic tweezers and discard.

Loosen with a second clean knife (knife no. 2) the abdomen muscle and lift it from the exoskeleton with a clean pair of tweezers.

Determine and note the sex by examining the gonads.

Transfer the muscle with a clean pair of plastic tweezers (5.8) into a preweighed plastic container (5.9), determine and note the fresh weight of the muscle. Close the container airtight, label it with the sample code, place a suitable number of containers in a plastic bag, add a sample identification note to the containers, and close the bag airtight and deep freeze the samples.

(b) Composite sample: Start sample preparation as described for single specimen sample above taking care to record length, fresh weight, tail muscle weight and the sex of each specimen separately. Reduce the tail muscle(s) of the large specimens to the weight of the smallest tail muscle. A composite sample should not contain less than 6 tail muscles from 6 different specimens of

the same sex and size. Homogenize the tail muscles in a blender (5.17). Transfer the homogenate into a suitable clean container (5.9) which has been weighed empty. Close the container airtight, label it and weigh the container with the homogenate. Note the weight of the homogenate together with the other data in a protocol. Place a suitable number of containers in a plastic bag (5.4), add a sample identification note, close the bag airtight and deep-freeze (5.3) the containers.

This represents the "tissue sample".

NOTE: The concentration of trace metals in a composite sample should represent the mean value of metal concentrations of single specimens. In order to avoid overrepresentation of large specimens, only shrimp of similar size (age) should be used for the preparations of composite samples. In addition, the weight of the tail muscles of all specimens to be included in the composite sample should be reduced to that of the tail muscle of the smallest specimen. As there might be considerable differences in the trace metal content of male and female specimens, use them in separate composite samples.

7.4 Sample preparation for small and medium size fish

(a) Single specimen sample: Determine the fork-length (from tip of snout when the mouth is closed to the apex of the fork of the tail) of fish (figure 4) to the nearest mm on the length-measuring board (5.5). Weigh the fish on a clean "weighing plastic" (5.11) with an accuracy of 0.1% of its total weight and note both the fork-length and the fresh weight of the specimen.

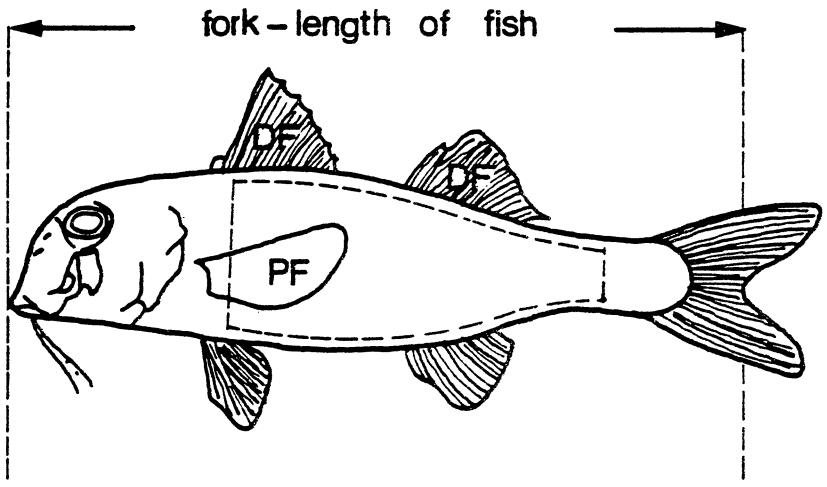

FIGURE 4 : SCHEMATIC DIAGRAM OF A FISH
(PF=pectoral fin, DF=dorsal fins, dashed line shows where the cuts should be made)

Rinse the fish with distilled water (4.1) or clean sea water (4.2) and place it on a clean working surface (5.7). Remove the pectoral fin and cut the skin of the fish with a first knife (5.6) near the dorsal fins, starting from the head to the tail (figure 4).

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 only taking care not to cut too deep in order to avoid cutting into the viscera and thus contaminating the fillet. It is advisable that a second person hold the fish by the head and tail during this operation.

Pull the skin from the fillet with a pair of tweezers (5.8), taking care that the outer skin does not contaminate the fillet.

With a second clean knife (5.6), cut the fillet from the vertebral column (backbone) starting from the cut near the gills. Lift the fillet with a second clean pair of tweezers (5.8), so that the fillet will not touch the working surface (e.g. the Pyrex dish) or other parts of the fish.

Weigh the fillet in a clean plastic container (5.9) and note its fresh weight.

If one fillet does not yield enough material for analysis, put the fish, skin side upwards, on a clean portion of the working surface (5.3) or on a new working surface and remove the second fillet from the other side of the same fish as described above, add it to the first sample and record their total weight.

Close the container airtight. Identify the container with a code number and/or label, record all data in the protocol and deep-freeze (5.3).

This represents the "tissue sample".

Determine and note the sex of fish by examining the gonads.

NOTE: Comparing the weight of the container holding the fillet sample(s) determined at this point with the weight of the container before the digestion step will show if the tissues have lost moisture during prolonged storage.

(b) Composite sample: Start sample preparation as described for single specimen sample above taking care to record the length, the fresh weight and fillet (sample) weight of each fish separately. Determine and note by examining the gonads the sex of each specimen separately.

Reduce the fillet(s) of the large specimens to the weight of the smallest fillet. A composite sample should not contain less than 6 fillets from 6 different specimens of the same sex and size. Homogenize the fillets in a blender (5.17). Transfer the homogenate into a suitable clean container (5.9) which has been weighed empty. Close the container airtight, label it and weight the container with the homogenate. Note the weight of the homogenate together with the other data in a procotol and deep-freeze (5.3) the container.

This represents the "tissue sample".

NOTE: The concentration of trace metals in a composite sample should represent the mean value of metal concentrations of single specimens. In order to avoid overrepresentation of large specimens, only fish of similar size (age) should be used for the preparation of composite samples. In addition, the weight of the fillets of all specimens to be included in the composite sample should be reduced to that of the fillet of the smallest fish. As there might be considerable differences in the trace metal content of male and female specimens, use them in separate composite samples.

7.5 Sample preparation of large-size fish

If necessary, thaw partially, e.g. overnight in a refrigerator (-2 to 4°C), the subsample taken in the field during sampling (6.4).

Rinse the subsample with distilled water (4.1) or clean sea water (4.2) and place it on a clean working surface (5.7). Remove any skin and bone that may be present. Cut off thin slices from all surfaces with clean plastic knife (5.6) and discard them. Repeat the operation with a second clean knife (5.6) in order to obtain a clean uncontaminated block of homogeneous tissue.

NOTE: It has been recognized that differences in trace metal concentrations may exist between different muscles in large fish, therefore as much information as possible on the actual sample should be recorded.

Transfer the tissue into an airtight container (5.9), close and label it, weigh it, note all data together with data of the subsample in the protocol, and deep-freeze (5.3).

This represents the "tissue sample".

8. SAMPLING AND SAMPLE PREPARATION PROTOCOL

Fill in the sampling and sample preparation protocol (table 1) giving full details in every column. This protocol should be attached to the test report on the determination of trace metals in the analyzed sample.

The following guidelines should be kept in mind when completing the protocol (the numbers refer to those used in table 1):

1.1 Use the scientific name for the species sampled. If necessary indicate subspecies or variety.

1.2 Indicate the name under which the species is known locally.

1.3 Use any code adopted by your institution. Never use the same sample code for more than one sample.

3.2 For samples obtained on fish market, indicate the town (village) where the market is. For samples taken at standard sampling stations or areas, indicate the name (code) of the station or area.

3.3 If the sampling point does not coincide with a standard sampling station or area, it may be advisable to code (name) it, in particular when the sampling point is used more frequently (e.g. a particular fish market). Never use the same sampling point code for more than one sampling point.

3.4 and 3.5 Always indicate the longitude and latitude of the sampling point to the nearest minute. For samples obtained from fish market, enquire about their provenience and try to reflect it also as geographic co-ordinates. Circle either E or W and N or S, as appropriate.

3.6 Give any additional information which may be relevant for the interpretation of the results (e.g. sampling point in vicinity of outfalls or similar).

4.1 Indicate the difference between data given under 2 and 5.

4.2 Mark the storage conditions used. If none of them applicable, give additional explanations in 4.3.

6.2 Identify sex of the specimen whenever possible. As for specimen length, determine shell length for mussels, fork length for fish and total length for shrimp as indicated in figures 2, 3, and 4. Specimen weight always refers to the fresh weight of the whole mussel, of the whole shrimp and of the whole fish. Note that sample weight, in the case of mussels, refers to the total weight of soft tissues. In the case of shrimp, the sample weight refers only to the fresh weight of the muscle, and in the case of fish, to the fresh weight of the fillet or of the combined weight of fillets removed from the same fish.

6.3 Whenever possible use six or more specimens of the same sex and size (age) in preparing composite samples. Mean length and weight refers to the arithmetical mean of the weight and length of individual specimens, as explained above. Always calculate the standard deviations.

Table 1: Sampling and Sample Preparation Protocol

1. Sample (specimen)

1.1 Scientific name:_____

1.2 Common name:_____

1.3 Sample code:_____

2. Date of sampling: day_____; month_____; year_____

3. Sampling point

3.1 Country:_____

3.2 Type of sampling point:_____fish market;

_____sampling area/station

3.3 Sampling point code:_____

3.4 Longitude: _____° _____' E or W

3.5 Latitude: _____° _____' N or S

3.6 Conditions at sampling point which may be relevant for the interpretation of results:

_____

_____

_____

4. Sample storage

4.1 Duration of storage:_____hours; _____days

4.2 Storage: deep-freezing_____; cooling_____

4.3 Factors relevant to sample storage which may be important for the interpretation of results:

_____

_____

_____

5. Date of sample preparation: day_____; month_____; year_____

6. Sample preparation

6.1 Tissue type (kind)_____

6.2 Single specimen sample: sex____; specimen length_____cm;

specimen weight_____g; sample weight_____g

6.3 Composite sample:

- number of specimens _____; sex_____
- mean length of specimens_____cm; stand. dev._____
- mean weight of specimens_____g; stand. dev._____
- total weight of composite sample_____g
- total net weight of homogenate_____g

6.4 Factors relevant to sample preparation which may be important for the interpretation of results:

_____

_____

_____

7. Full address of the institution carrying out the sampling and sample preparation:

_____

_____

_____

_____

8. Name(s) and signature(s) of the person(s) who carried out the sample preparation:

_____

_____

Date:_____

Appendix A

Preparing plastic tweezers

Methylmetacrylate of 4 mm thickness has been found to be very useful as it has 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 can be used also. However, it is much more difficult to make tweezers by heating the plastic in an oven since the plastic twists easily.

Materials:

- sheets of acrylic (methylmetacrylate) resin; 4 mm thick (trade names: e.g. Perspex, Flexiglas, Lucite);
- a plastic tube, about 40 mm in diameter.

Equipment:

- hot air blower (300-350°C) used for molding plastics, or Drying oven (135-140°C).

Procedure:

(a) With a hot air blower

- cut from the sheet with an electric or a hand saw strips of about 10 mm width and 250 mm length;
- heat about a 60 mm long part in the middle of the strip so that it bends easily. Bend it around the plastic tube carefully in order to make both ends meet. Cool the plastic with cold water;
- sharpen the ends with a file and roughen the inside of the tweezers so that they grip well;
- wash the tweezers carefully with detergents and rinse them with distilled water.

(b) With a drying oven

- place the plastic strip on a clean piece of wood in a drying oven (135-140°C) until it becomes soft;
- lift the strip at one end with a pair of tweezers and bend it around the plastic tube without letting the tweezer tips meet;
- cool the tips by dipping them in a beaker of clean cold water and afterwards bend the ends of the tweezer so that the tips meet;
- prepare the ends of the tweezers as described earlier.

Appendix B

Sample indentification note

A standard sample identification note should contain the following data:

- sample code (the same code should be used in 1.3 of the Sampling and Sample Preparation Protocol; see table 1);
- species name (important in particular whenever storage of sample may create difficulties in determining the species);
- sampling date;
- sampling location (given as sampling point code, if possible; see 3.3 of table 1);
- collector's (sampler's) name;

Example:

AN 435
Mytillus galloprovincialis
3 March 1982
F 17
D. Degobbis

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