TOWARDS A STANDARDIZATION OF MEDITS BENTHOS PROCESSING: SOME PROPOSALS FOR A COMMON PROTOCOL

Daniela Massi, Antonino Titone, Cinzia Badalucco, Danilo Scannella, Fabio Falsone, Michele Geraci, Federico Di Maio

Institute for Marine Biological Resources and Biotechnology (IRBIM)
National Research Council (CNR)
Via Vaccara, 61 – 91026 Mazara del Vallo (TP), Italy
daniela.massi@cnr.it

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INTRODUCTION

MEDITS trawl surveys represent a unique large-scale and long-term source of spatial data on benthic species and communities in the Mediterranean.
Despite limitations due to relatively low and biased catch efficiency of bottom trawls, these data are receiving increasing attention within the framework of EAFM (Ecosystem Approach to Fisheries Management) and conservation programmes. However, to date no harmonised procedure for sampling and processing of benthos exists in the MEDITS community.

This presentation summarizes experiences gathered, since 2004, in sampling and processing procedures developed at CNR of Mazara del Vallo Laboratory for benthos studies including MEDITS program. Some critical issues faced during sampling and processing are presented. Our work could contribute to the discussion for achieving agreed procedure of a standardized protocol to be adopted at voluntary basis for analyses on benthic organisms.

WHAT IS FORESEEN ACCORDING TO THE MEDITS HANDBOOK (2017)

Taxonomic categories

• From 1994 only the categories A “Fish”, B “Crustaceans”, C “Cephalopods”, D “other commercial species” and E “other animal species but not commercial” were covered;

• From 2012 all the subcategories with the addition of the categories “G portions or products of animal species”, “H …of vegetal species“ and “V plantae“ have been introduced;

• From 2014 the last categories of marine organisms of M Mammalia, O Aves and R Reptilia are introduced.

Treatment of the catch

•According to the MEDITS Handbook (2017), the megabenthic fraction of catches is considered as benthic organisms larger than 1 cm;

• For each species the total weight and number of individuals should be collected, excluding the taxonomic category V, G, H for which only the total weight should be collected;

• For taxonomic categories D and E the number of individuals is not mandatory;

• The sub-sample should be not less than 100 individuals.

The Mazara del Vallo Laboratory, according to the MEDITS Handbook (2017) contributed to the MEDITS TM List with about 629 new species, 81 out of this 629 species recorded in GSA 16 only.

PROCEDURES ADOPTED IN GSA 16

Sampling on board

Samples of benthos are collected by haul, in plastic bags, frozen at -40 °C and stored at -20 °C.
Routinely the total catch is taken. In case of particularly abundant catches the total weight is recorded and a representative sub-sample of a maximum weight of 5000 g is taken, noting its weight.
Species of sure taxonomic identification, such as some non-target crustaceans (*Geryon, Bathynectes, Polycheles* and so on) can be directly processed on-board in terms of total number and total weight by species.
Rare species or species under specific investigations are stored separately in labeled bags for more in depth analysis at Laboratory.

Sub-sampling procedures

The samples may have been sub-sampled on board assigning a first-level raising factor (RF1°)
Sometimes, in the laboratory, it may be necessary to further sub-sample in order to reduce the enumeration of the species so a second-level raising factor will be assigned (RF2°)
Seldom, it is possible that a further sub-sampling is necessary for some species (for example in the case of abundant crinoids) and a third-level raising factor will be applied (RF3°)

PROCESSING AT THE LABORATORY

Frozen sample thawing occurs gradually over a period of 12-24 hours at room temperature and can be facilitated by running cold water in a perforated container, which can help the water spill.

Sorting and identification of sampled organisms is carried out at the most detailed possible taxonomic level (species). If the identification is uncertain the collaboration of specialists is asked.

Before being weighed, all individuals absorbing water (Sponges, Tunicates, etc ...) and/or sediment (*Actinauge richardi*), must be treated mechanically (by squeezing) in order to allow water to escape and/or sediment removal.

In the Mazara del Vallo Laboratory the collected information for each species is reported in the ad-hoc form (Table 1). It can be useful to note any interesting information using the "Notes" field of the form.

Table 1 – Form reporting the collected information on benthos used at CNR Laboratory of Mazara del Vallo.

Annex I - MEDITS BENTHOS

TRAWL SURVEY

HAUL DATE DEPTH

SPECIES CODE	SCIENTIFIC NAME	N°	WEIGHT (g)	RF 1°	RF 2°	RF 3°	NOTES

The procedures adopted in the Mazara del Vallo Laboratory and some critical issues in samples processing are briefly described below and summarized in Table 2.

Firstly, it is important to underline we adopted the approach that the organisms should be classified at the most detailed level possible, the species, and that they should be included into the categories already present in the MEDITS Handbook (2017).
We propose new sub-categories, written in bold in Table 2, in order to classify everything that is collected from the bottom trawl during the hauls for better use of the MEDITS data in the perspective of the EAFM and main European Policies concerning the marine ecosystems.
Fishes, Cephalopods and target species of Crustaceans are excluded from the analyses.

Intact organisms

- For animal taxa, categories B, D and E, Number (N) and Weight (W) are recorded, reporting them in 1 row (record) for each identified taxon, even if for D and E the N is not mandatory
- For plant taxa, category V, only W is recorded

Broken non identifiable organisms

Broken organisms are those lacking large parts of the body. Currently they are considered Biological debris but they should be divided into new 2 sub-categories of the existing categories G and H for which only W is recorded:
- Debris of marine fauna (to be codified as Gdmf)
- Debris of marine flora (to be codified as Hdmf).

Broken identifiable organisms

They are classified as Biological debris (categories G or H), if there are only a few fragments, of which only the W is collected.

If they are broken but a large part of the organism is present, N and W are collected and eventually added to the N and W of the intact individuals of the same taxon present in the sample.
In particular, the following rules are adopted according to the special cases:
- Colonial individuals: almost all the colony
- Crustacea: with at least the cephalo-thorax or carapace
- Echinoidea: at least 50% of the shell and digestive system
- Ophiuroidea: with at least the entire central body
- Asteroidea: with the central body and half of the arms + 1
- Crinoidea: with at least the central calyx

Empty shells

The organisms with an intact shell but without mollusk are considered Shell debris and are already codified in the TM List, but to know better the thanatocoenosis, we divided the existing Shell debris of the category G into the new sub-categories to be codified:
- Gastropoda debris (to be codified as Ggd)
- Bivalvia debris (to be codified as Gbd).
Furthermore, we also record the name of the species if identified, for example: “Bivalvia debris - Neopycnodonte cochlear”, recording W only, while the N of the empty shells are reported in the Notes.

Aggregations

The "aggregation" of organisms or two or more organisms belonging to different taxa and/or parts of these closely linked, are processed according to the following approach:
- If Aggregations are separable and identifiable (for example gastropod-hermit crab-actinia) the N and W are recorded for each identified taxon
- If Taxa are not separable but identifiable only the W is recorded by extrapolating it as % of the total W of the aggregation
- If Aggregations are not identifiable, not separable and calcareous, such as Bioconstruction, only the W for the entire category is recorded. This sub-category need to be coded.

Eggs Capsules

In the case of eggs capsules we proceed as follows:
-egg capsules belonging to Elasmobranchs are numbered, weighed and sub-categorized at lowest taxon is possible and codified as "Debris-eggs capsule -Taxon name";
-eggs aggregation belonging to Gastropods and Cephalopods are weighed and sub-categorized at lowest taxon is possible and codified as "Debris-eggs aggregation -Taxon name".
Some eggs capsules in the TM List are already coded for example of *Scyliorhinus* and of Rajidae among the Selachians. Since it is possible to distinguish the eggs capsules of *Galeus* we suggest to codified also them.

Other Debris

Other new debris categories which are recognized in our Laboratory and could be codified are:
- Terrestrial organic debris (trunks, leaves), useful as a transport index, in which only W is recorded
- Inorganic debris (rocks, lava, coal), useful as an index of the nature of the bottom, in which only W is recorded
- Anthropic waste (nets, plastic, knives, cans etc.) are numbered, weighed and identified according to the Protocol for Monitoring of Marine Litter.

REFERENCES

MEDITS-Handbook, (2017). Version n. 9, MEDITS Working Group: 106 pp.

Table 2 - Towards a standardization of benthos processing: categories and data collected proposed for a Common Protocol. The new sub-categories proposed are in bold.

CATEGORY	N°	WEIGHT (g)	INPUTTING FORMAT
INTACT ORGANISMS			
• Animals *Taxa*: solitary or colonial (B,D,E)	X	X	1 record for each *Taxon* identified
• Plants *Taxa* (V)		X	1 record for each *Taxon* identified
BROKEN NON IDENTIFIABLE ORGANISMS			
Biological debris, if possible to split into:		X	1 record for the entire category
• "**Debris of marine fauna**" (G)		X	1 record for the entire category
• "**Debris of marine flora**" (H)		X	1 record for the entire category
BROKEN IDENTIFIABLE ORGANISMS			
Biological debris G or H: if few fragments		X	1 record for the entire category
Broken, but with almost the whole body. In particular the special cases:	X_{extr}	X	1 record for each *Taxon* identified. To be added to N. and W. of intact individuals of same *Taxon.*
• Colonial individuals*:* almost all the colony	X	X	1 record for each *Taxon* identified
• *Crustacea:* with at least cephalothorax or carapace	X	X	1 record for each *Taxon* identified
• *Echinoidea:* at least 50% of shell and digestive system	X	X	1 record for each *Taxon* identified
• *Ophiuroidea:* with nearly entire central disc	X	X	1 record for each *Taxon* identified
• *Asteroidea*: with central body and ½ + 1 whole arms	X	X	1 record for each *Taxon* identified
• *Crinoidea:* with nearly central calyx	X	X	1 record for each *Taxon* identified
EMPTY SHELLS			
"Shell Debris" (G), if possible to split into:		X	1 record for the entire category
• "***Gastropoda*** **Debris**"		X	1 record for each *Taxon* identified
• "***Bivalvia*** **Debris**"		X	1 record for each *Taxon* identified
AGGREGATIONS			
• Separable and identifiable aggregations	X	X	1 record for each *Taxon* identified
• Identifiable *Taxa* but not separable		X extrapolating the weight% of the *Taxon* on the total	1 record for each *Taxon* identified
• "**Bioconstruction**": non-identifiable and non-separable calcareous *Taxa*		X	1 record for the entire category
EGGS CAPSULES			
"Eggs capsules of *Scyliorhinus"*	X	X	1 record for every *Taxon* identified
"Eggs capsules of *Rajidae"*	X	X	1 record for every *Taxon* identified
"**Eggs capsules of *Galeus***"	X	X	1 record for every *Taxon* identified
"Eggs capsules *Gastropoda"*		X	1 record for every *Taxon* identified
"Eggs capsules *Cephalopoda"*		X	1 record for every *Taxon* identified
OTHER DEBRIS			
"**Terrestrial organic debris**"		X	1 record for the entire category
"**Inorganic debris**"		X	1 record for the entire category
"Anthropogenic waste"	X	X	according to the Protocol for Monitoring of Marine Litter