Autonomous Reef Monitoring Structures (ARMS)

Benthic marine fauna processing manual

BDMY
BioDiversidad
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What are ARMS?

Autonomous Reef Monitoring Structures (ARMS) are standardized 3D collectors of marine life. They are stacks of plates that mimic the complex structure of the sea bottom that is hard to sample without destroying natural habitat.

What is the objective of ARMS?

The aim of these structures is to standardize the assessment and monitoring marine biodiversity, to document marine communities around the world.

"ARMS act as biological weather stations that are deployed for a period of time, then retrieved and disassembled by a team of experts to observe local species recruitment and analyze diversity allowing researchers to compare one place to another or how a place changes over time".

Why are scientific collections important?

AMERICAN MUSEUM OF NATURAL HISTORY

Collections provide the evidence from which scientists derive scientific knowledge, including knowledge that is directly applied to critical issues facing our society, such as:

1. Documenting biological and cultural diversity in a time of unprecedented environmental destruction
2. Developing a baseline understanding of the effects of climate change and other environmental threats
3. Monitoring the changes in marine resources

Also, they are a libraries of **biodiversity**, collections also help establishing species identities.

What is this manual?

In order to analyze the biodiversity of coral reefs, specifically benthic marine fauna, this manual was developed by a group of taxonomic experts to guide the proper treatment and preservation of organisms associated with autonomous reef monitoring structures (ARMS), and thus for their inclusion in scientific collections.

Content

Material

	• 15 to 20 rectangular white trays of 5 liters (43.5 cm x 28.5 cm x 8 cm) per ARMS
	• 1 Nut driver¼
	• 1 Spanner¼
	• Camera, reflex camera preferably, to improve the quality of the pictures
	• Standard lens for a reflex camera (18-55mm)
	• Macro lens for a reflex camera (optional)
	• Tripod
	• Flash (optional)
	• Waterproof labels for each plate of ARMS device. The label codes must indicate the location (the place where the structure comes from), ARMS structure number (if there is more than one structure to be processed), plate number (1-9), and orientation (face and back). Example: Yuc_ARMS1_P1_CA
	• Waterproof labels for samples, numbered 01 to 50, depending on the number of samples per ARMS. The labels must follow the code of each structure and sample: PLACE-YEAR_ # ARMS_001 (Example: Yuc2020_ARMS2_001)
	• Logbooks (In appendix)
	• Nitrile gloves
	• 4-5 White light lamps
	• Bottle with 4% formaldehyde in seawater Wash
	• Bottle with 7-10% formaldehyde with seawater
	• Bottle with 96% ethanol

	• Bottle with 70% ethanol
	• 50 ml of a saturated solution of menthol crystals and 96 ° ethanol
	• 100 g of sodium chloride dissolved in 1 of liter seawater
	• 100 g of magnesium chloride or sulfate dissolved in 1 liter of seawater
	• 40 liters of seawater
	• 2 Dissection needles
	• 2 INOX Gouges
	• Sieve with a mesh opening of 500 µm (It is recommended to use a sieve for physical tests INOX)
	• Four 4oz / 113g fishing sinkers or any material heavy enough to submerge the plate in the photography process and avoid reflections
	• Glass storage containers for samples (1-8oz glass jars are preferable for long storage specimens, approximately 200 units)
	• 100 microtubes for molecular samples
	• 10 ml of clove oil or eugenol diluted in 100 ml of seawater
	• 10 Tweezers
	• Fine paintbrushes
	• 30 Petri dishes of 60, 90, and 100 mm
	• 21.5 x 28 cm of filter paper (without specific thickness)
	• 2 Scalpel
	• Data backup device (hard disk, flash drives)
	• 1 Hammer

	• 1 liter 10% sodium hypochlorite (commercial chlorine) and / or 1 liter of 0.5% boric acid
	• Bouin solution (See obtaining procedure: https://mmegias.webs.uvigo.es/6-tecnicas/protocolos/s-fijador-bouin.php)
	• 10 tongue depressors
	• 10 rubber bands
	• 1 dropper
	• 2 Scissors
	• Heating grill

Material catalogue

White rectangular trays (15-20)

Nut driver ¼ (1)

Spanner ¼ (1)

Camera (1)

Standar and macro lens

White light lamp (4-5)

Tripod (1)

Paintbrushes (3-4)

Dissection needles (2)

INOX Gouges (2)

Sieve (1)

Fishier sinkers (4)

Storage containers for simples (100)

Microtubes for molecular simples (100)

Tweezers (10)

Petri dishes (30)

Filter paper

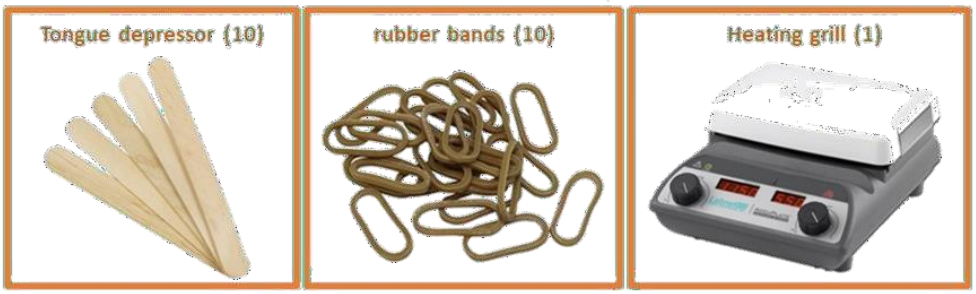
Scalpel (2)

Hammer (1)

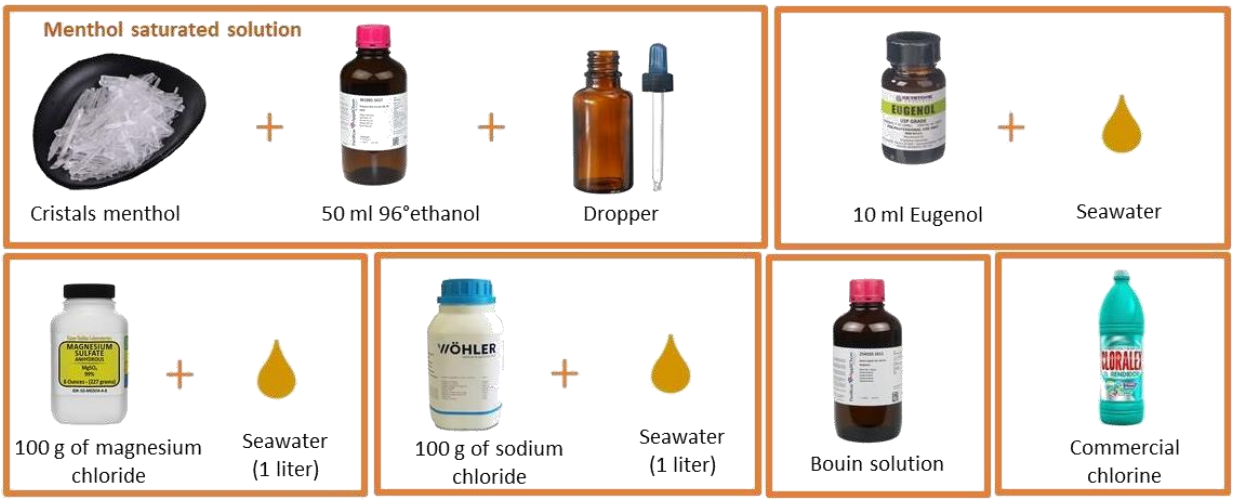
Dropper (1)

Scissors (2)

Wash bottle (5)



Solvent's catalogue



Specimen procedures and photography

The structures must be transfered to the laboratory with air pumps and seawater. It is important to keep the structures in a temperate place, always keeping the aeration pumps. It is also necessary to bring at least 40 liters of filtered or unfiltered seawater to the laboratory (for each ARMS structure) from the same locality where the structures come from. It is recommended to process the samples immediately or less than 24 hours after the recovery of the structures.

Nine consecutive trays will be placed on the table to process the samples, each one with the label of each plate (Face and/or Back) and two to three liters of seawater, enough to cover the plate. Two extra trays must be kept for processing mobile fauna; one with sodium chloride for echinoderms and mollusks; and the other with clove oil for crustaceans.

Once the tensioners and the basket have been removed (it is recommended to examine the basket for mobile fauna), the ARMS structure can be disassembled. Each plate of the structure will be placed in one of the previously labeled trays with seawater (**Fig. 1**).

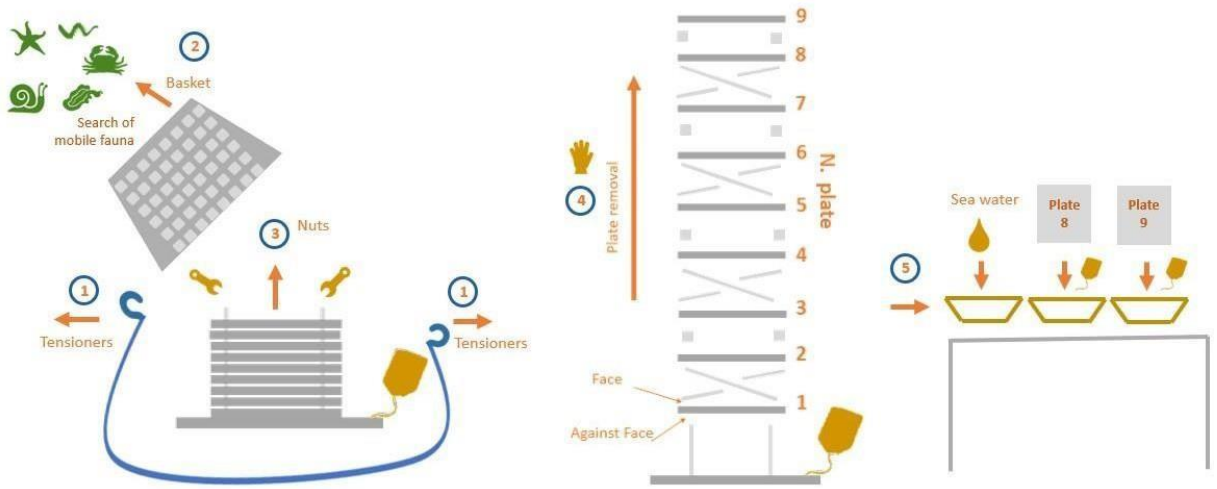


Fig. 1 Procedure for plate preparation and separation of fauna. 1) Removal of tensioners, 2) Removal of protective basket and collection of mobile fauna, 3) Removal of nuts with Spanish key, 4) Removal of plates with numbering andorientation (it is recommended to use gloves), 5) Placement of plates in trays with sea water.

Separation of sessile and mobile fauna

After placing the plates on the trays in an orderly manner, proceed to carefully sort the mobile fauna. This procedure is done for each plate and for the container and basket where the structure was contained (white light lamps are recommended for easy observation of the organisms). Once the taking of photographs has finished, proceed with separating the sessile fauna from each plate. Subsequently, the seawater is filtered from each tray and the container where the ARMS structures were transported using the sieve. The fauna retained on the sieve is preserved in 70% ethanol (**Fig. 2**).

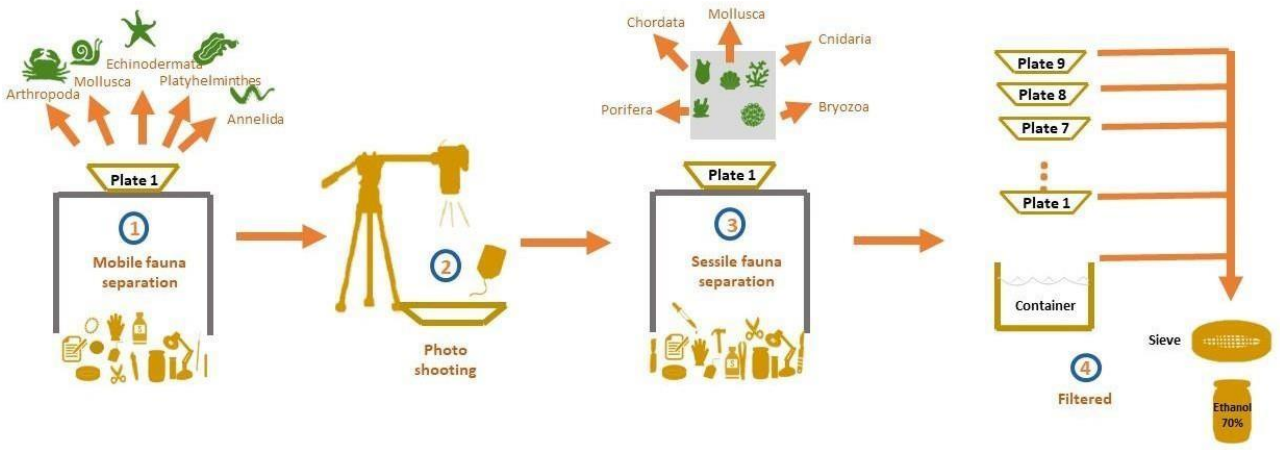


Fig. 2 Monitoring of activities for the processing of recruitment plates and marine fauna. 1) Separation of mobile organisms with the help of lamps, 2) Photography of plates on both sides for sessile organisms, 3) Separation of sessile fauna with the help of lamps.

Taking photographs of plates (sessile fauna)

Each tray with the plates will be placed on a flat surface to be photographed. For this process, a camera with an 18-55 mm lens will be used, programmed with the timer option, and supported by a tripod. Because the plates have positive buoyancy, a fishing sinker will be placed at each end of the plate to avoid buoyancy and reflections and favor the natural tension of the organisms on the plate (it is recommended to use flash linked to the camera in each side of the plate to decrease shadows and increase sharpness, as well as the manual mode of the camera to adjust the ISO and aperture). Two to three photographs will be taken per plate on each side; ensuring that the first photograph contains the label at the center (**Fig. 3**).

Once the photographic record of the plates has been made, the pictures must be back-up in storage devices (it is suggested to carry out the labeling by photography following the code of each plate, orientation, and ARMS number). After the photographing process, proceed by separating the sessile fauna in the plates on each side(it is important to note the plate number and the orientation of the sessile specimens).

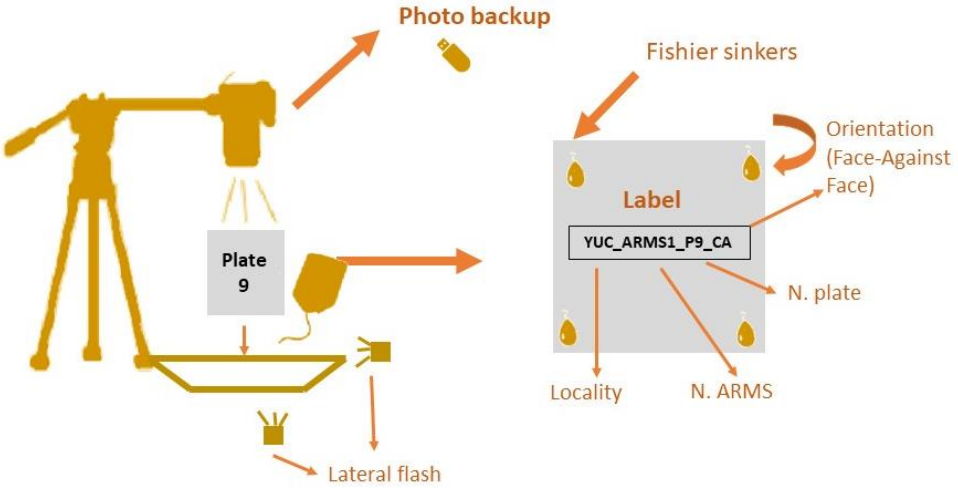


Fig. 3 Arrangement of tools for taking pictures of ARMS plates. Position of the tripod with respect to the plates and position at the ends of the fishing sinkers for the sinking of the plates and orientation of the labeling for identification ofthe photographs.

Processing of benthic marine fauna

Phylum Arthropoda

Superorder Eucarida

It is necessary to separate crabs, shrimps, and stomatopods carefully with watchmaker's tweezers to prevent the detachment of structures. Each individual will beimmersed in clove oil diluted in sea water until they achieve numbness (20 min).

In the case of hermit crabs, once anesthetized, it is advisable to remove them from their shell. For this it is necessary to remove the individuals from the cephalothorax (to avoid damaging pereiopods) carefully with tweezers slowly following the growth of the shell and to hold through the middle of the body until completely removing the abdomen of the crab (to facilitate the process, the crabs can be frozen after relaxation).

Regarding crabs, the specimens should be photographed in dorsal and ventral positions, for shrimp and stomatopods, the specimens should be photographed in the dorsal and lateral positions. All specimens should be preserved in containers with 70% ethanol and take a pereiopod for molecular studies in 96% ethanol% (**Fig. 4**).

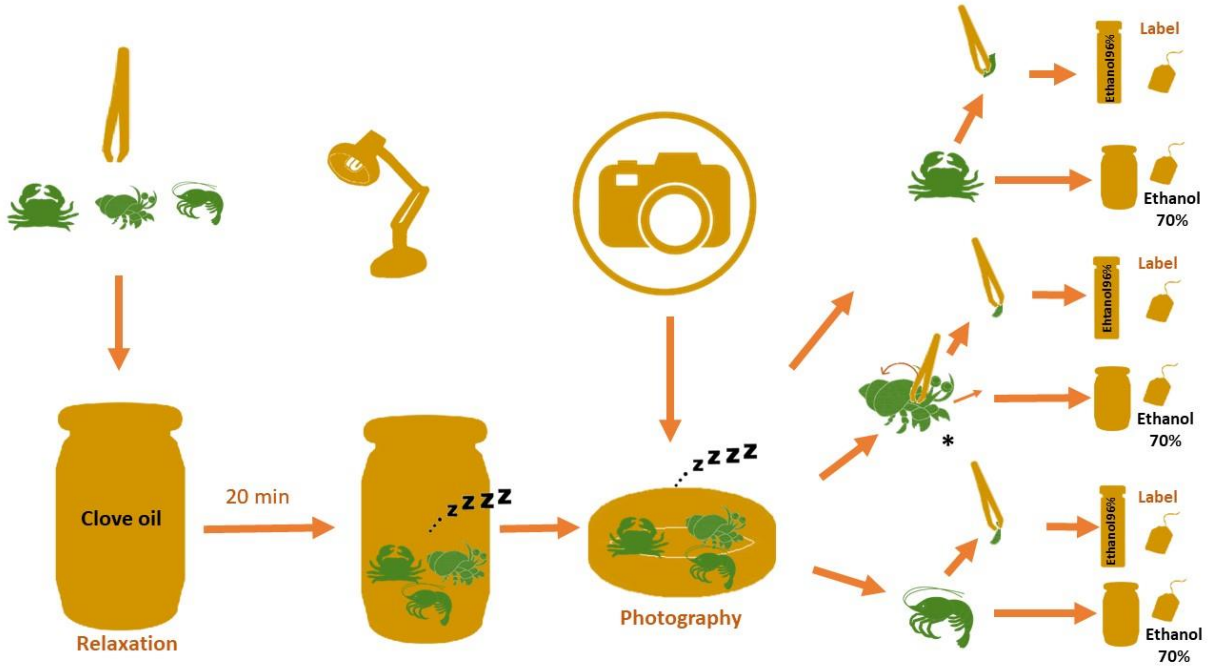


*Fig. 4 Processing sequence for the incorporation of specimens into scientific collections and sampling for molecular studies of crabs, shrimps, and stomatopods. * Separation process of extraction of hermit crabs from the shell*

Superorder Peracarid

During the seawater filtration process of trays and container of ARMS with the 500 µm sieve, the organisms are retained from the sieve lumen, which must be recovered withthe help of the 70% ethanol squeeze bottle and tweezers (be careful not to damage the organisms as they easily lose limbs). Specimens are stored in glass containers with 70% ethanol(**Fig. 5**).

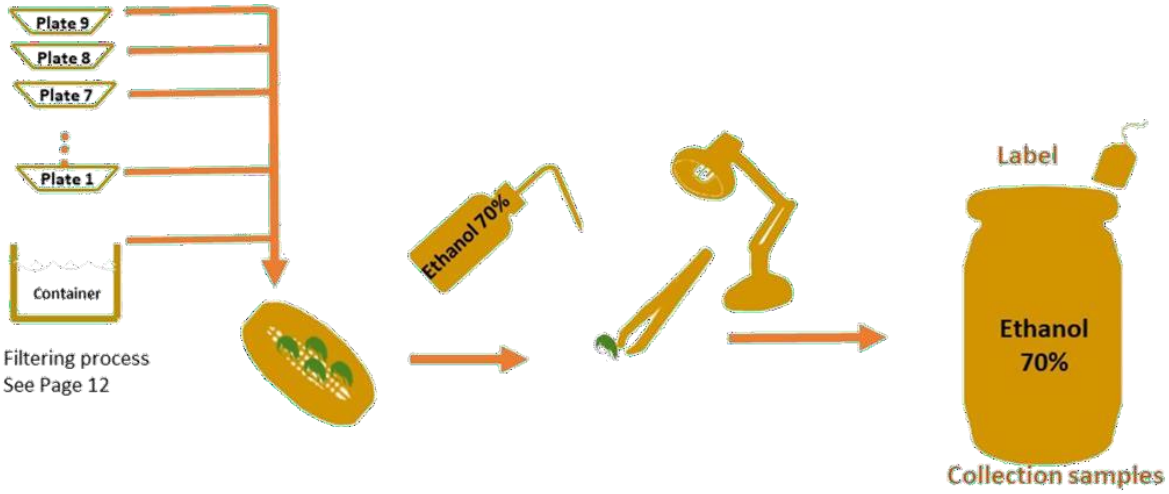


Fig. 5 Peracarid sample separation process by filtering water from ARMS trays and container.

Phylum Mollusca

Before being anesthetized for preservation, all the specimens of mollusks such as sea slugs, the soft part of the gastropods (head and foot), the mantle of the bivalves (for the species that have it), as well as the plates and the belt of the chitons should be photographed using a macro lens or a photographic camera adapted to a dissecting microscope, to record the original colors of the specimens because it is important for their identification.

Mollusks anesthesia consists in immersing the specimens in seawater with 10% magnesium chloride for 40 minutes. Gastropods with operculum or bivalves that are closed will require more time since they will be closed, preventing the penetration of the anesthetic solution. The time of anesthesia will depend on the size of the organism; larger animals will require more time. To confirm that animals are anesthetized, they will not respond to any tactile stimulus.

After the relaxation process, all individuals are sorted by species or morphotypes and preserved in 70% ethanol inside crystal containers. In the case of chitons of more than 10 mm, it is necessary to fix them carefully on tongue depressors supported with thin rubber bands for theircorrect stretching. This procedure allows the animals to remain stretched during anesthesia. Chitons less than 10 mm are placed in a small container and pressed while 70% ethanol is poured over them (10-15 seconds) to ensure that the specimen is extended after fixation and finally placed in containers. Sea slugs are carefully "slided" down per oneside of the container with 70% ethanol. For molecular studies, it is suggested to preservewhole organisms in 96% ethanol (**Fig. 6**).

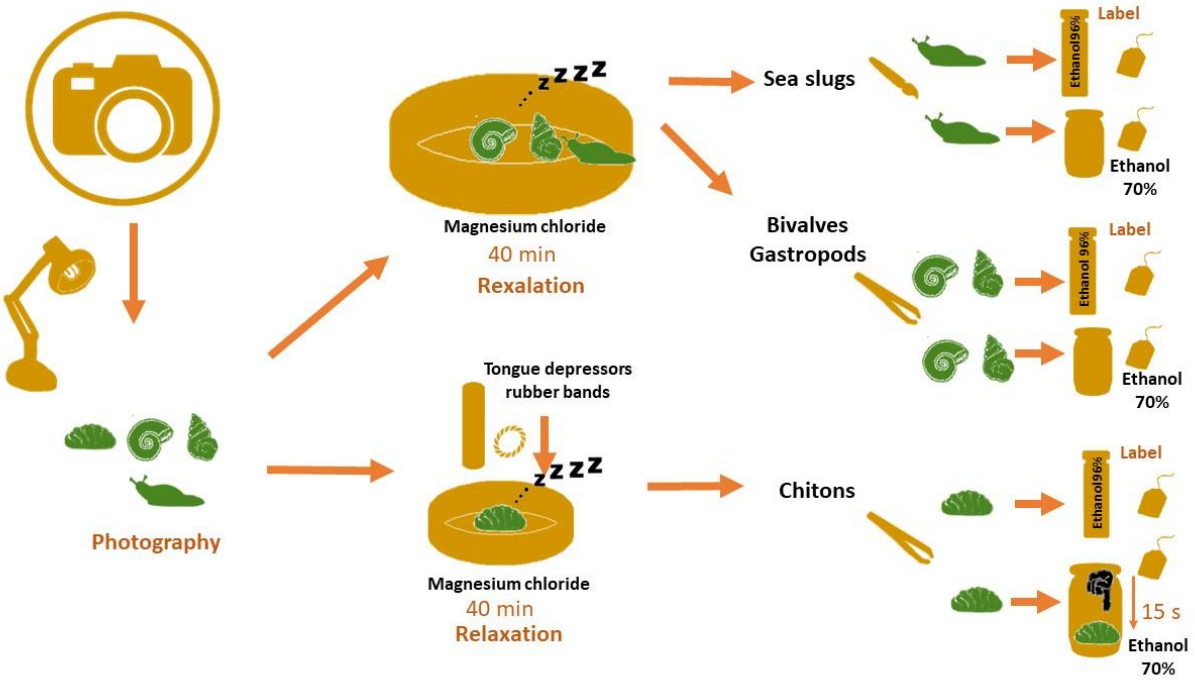


Fig. 6 Mollusks processing sequence per class for obtaining samples for molecular studies and preserving specimens forscientific collections.

Phylum Echinodermata

Most echinoderms can be manipulated by hand, while small individuals need to be manipulated with fine tweezers. Handling of sea urchins with sharp spines requires caution, as the spines and pedicels of some species are toxic. Ophiurids and crinoids are more delicate, and their specimens should be handled with care to avoid autotomy (fragmentation, loss of arms, evisceration). The handling should always be close to the center of the body (do not manipulate from the ARMS to avoid autotomy).

It is necessary to anesthetize the echinoderm specimens with chloride magnesium sulfate, or cold water (with ice) to avoid autotomy or the contraction of the tentacles (sea cucumbers) and tube feet (ophiurids and starfish). The anesthetic procedure starts when the specimens must be submerged in an isotonic solution of seawater and magnesium chloride or sulfate, covering the tray to prevent any entry of light during the anesthesia period of 15 to 45 minutes or until the specimens show no movement. It is recommended to take photographs with a macro lens or using a dissection microscope before fixing the specimens; these pictures will be evidence of their in vivo coloration, which later will facilitate taxonomic identification. Once the specimens are anesthetized, it is necessary for the ophiurids to be placed in a "kite" position, which allows adequate storage and subsequent observation of taxonomically important structures. To do this, the disc is gently held, and the organism is slid on a thinbed of 70% ethanol, and with the help of a dissecting needle, the ARMS are directed towards the opposite direction, following one direction only. It is important to consider that these specimens will remain rigid, and the final position will be permanently fixed.

For sea cucumbers, it is necessary to immerse them in 70% ethanol, holding them by the mouth to prevent the contraction of their tentacles when they enter in contact with the ethanol. As for sea urchins and starfish, after their anesthesia, they are placed directly in 70% ethanol. For molecular studies, it is suggested to preserve whole organisms in 96% ethanol (**Fig. 7**).

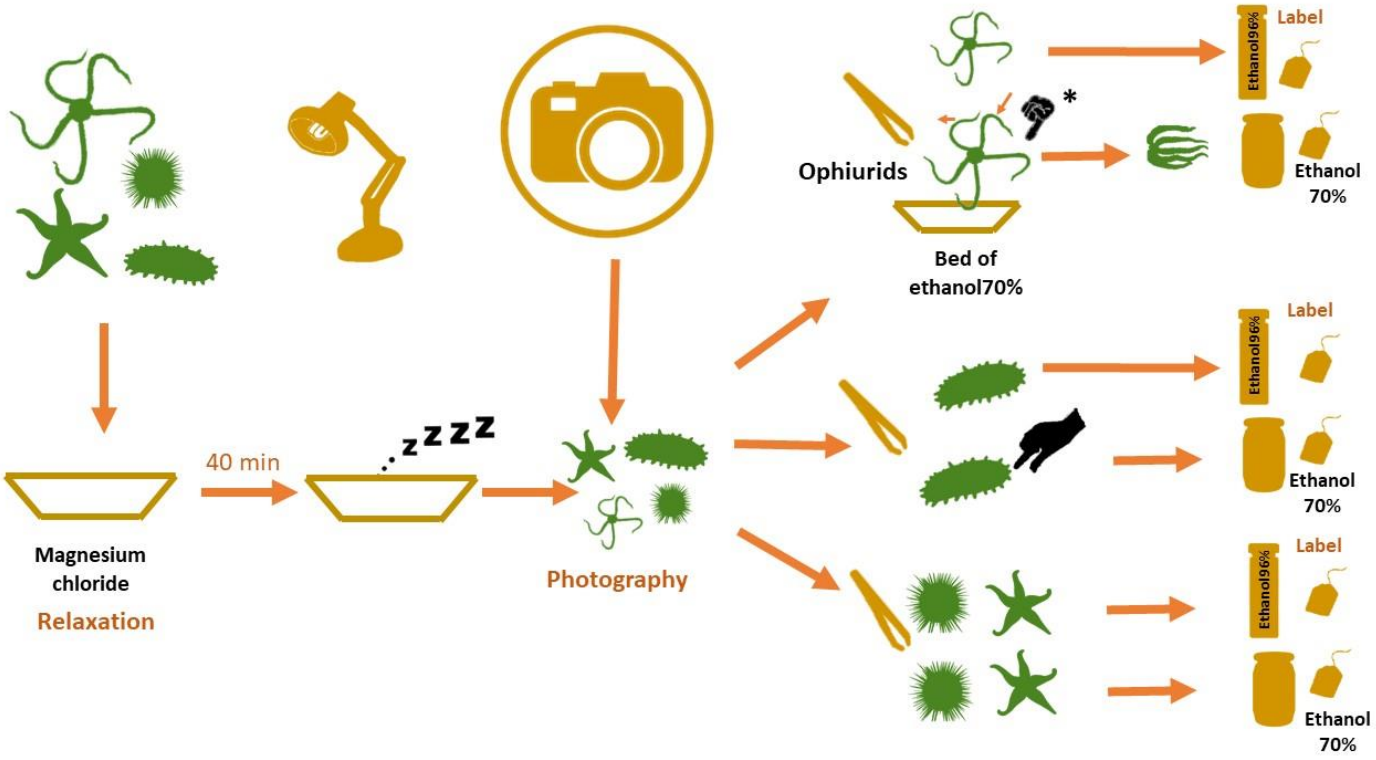


*Fig. 7 Processing for relaxation and preservation of echinoderms per class. * Note the proper "kite" position of an ophiuro for preservation and storage.*

Phylum Annelida

Class Polychaeta

Separate the organisms from the tray and carefully place them in containers with low temperature seawater (16°C) for at least 30 minutes in order to favor specimens relaxation and eversion of proboscis, extension of parapods and anterior structures. Taking photographs of the living organisms is recommended for having proof of it actual color. Once the specimens are relaxed (the organisms have slow movements) and with help of fine tweezers (avoid squeezing the specimens to prevent damage or detaching fragments such as palps, cirrus or chaetes), each specimen or specimens of the same species must be placed in 4% formaldehyde to fix them and include the specimen's label. After a 24 hours fixation, the formaldehyde should be replaced by 70% ethanol.

The material required for molecular analysis (it is recommended to preserve an entire organism or a small part of the final portion of the individual) will be directly preserved in 96% ethanol, without formaldehyde fixation (Fig. 8).

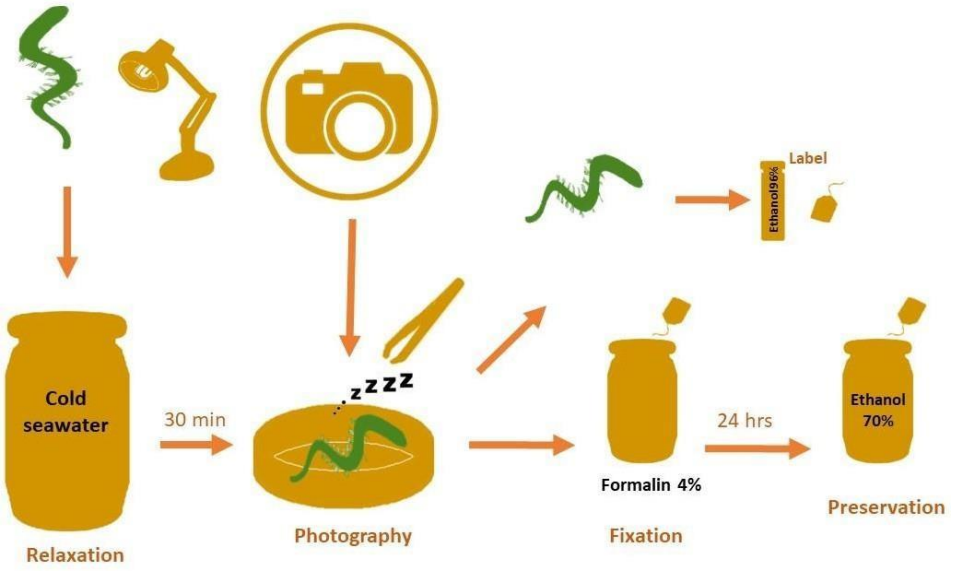


Fig. 8 Processing sequence of marine polychaetes for preservation and incorporation into scientific collections and molecular studies.

Phylum Platyhelminthes

After taking photographs of the individuals to record their coloration, it is necessary to relax the organisms by placing them in seawater at 4° C for at least 15 minutes, taking a small fragment for inclusion in 96% ethanol. At the same time, it is necessary to freeze 4% formaldehyde with seawater at half the capacity of a petri dish to the point of solidification.

A piece of filter paper is immersed in seawater, carefully placing the animal on the wet paper with the help of a fine paintbrush (this brush should only be used for seawater, as another brush will be used for the next step). Wait patiently with the submerged paper until the animal has crawled onto it and is fully stretched. After that, the piece of paper with the animal on top is placed on the frozen formalin plate and with a different paintbrush, spread the organism carefully in case it is not fully extended. Leave the animal in formalin for 3 to 5 hours. Finally, with a fine tweezer, remove the animal carefully and place it into a container with 70% ethanol for its preservation and further histology processes (**Fig. 9**).

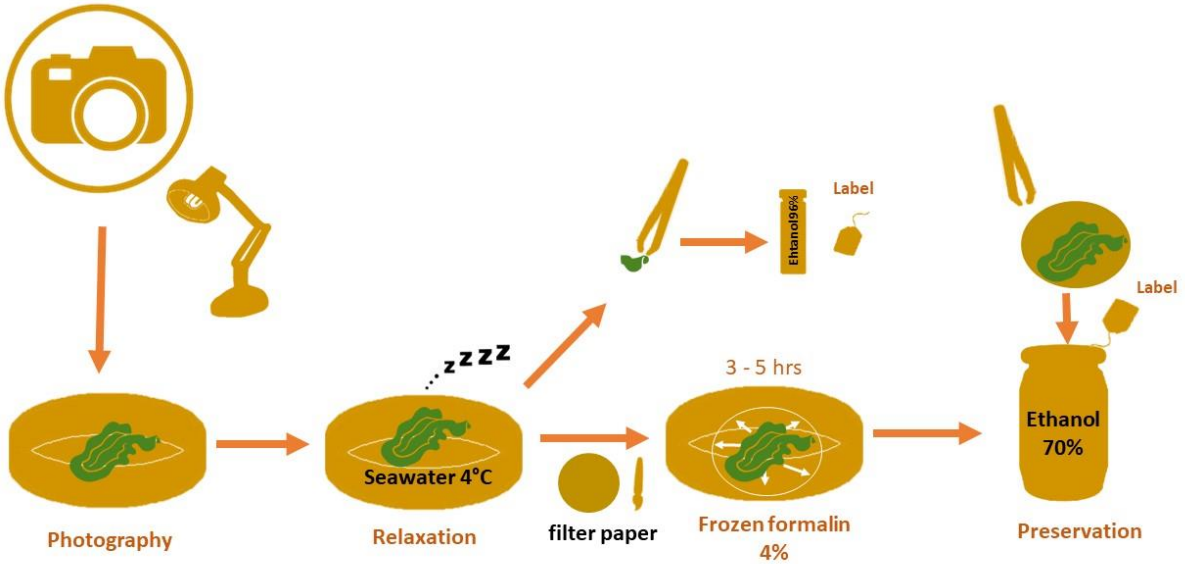


Fig. 9 Processing sequence for the preservation of flatworms.

Phylum Bryozoa

For the bryozoan colonies, it is necessary to separate two fractions of each colony. One fraction will be used for molecular studies by taking living tissue from it while the other sample will be used for the preservation of the morphological-anatomical structures of the colonies that later will be observed by Scanning Electron Microscopy (SEM) photographs.

For encrusting bryozoans, the colonies must be removed with the help of a gouge, applying pressure below the colony to obtain a fraction of 5 to 10 centimeters (generally the colonies belonging to the same species share the same color pattern and have radial growths) and in the case of the arborescent colonies, these are removed with the helpof fine tweezers and preserved directly in 96% ethanol.

For the observation of morphological-anatomical structures, one of the fractions of each colony is placed on a tray to allow it to dry at room temperature for 30 minutes. While the colony goes through the drying process, it is necessary to boil 1 liter of tap water and once the water is at its boiling point, add 200 ml of 10% sodium hypochlorite and letit boil for five more minutes. Subsequently, the colony fraction will be left to boil for 3 minutes and finally, it should be removed and rinsed with running water, left to dry for 24 hours (**Fig.10**).

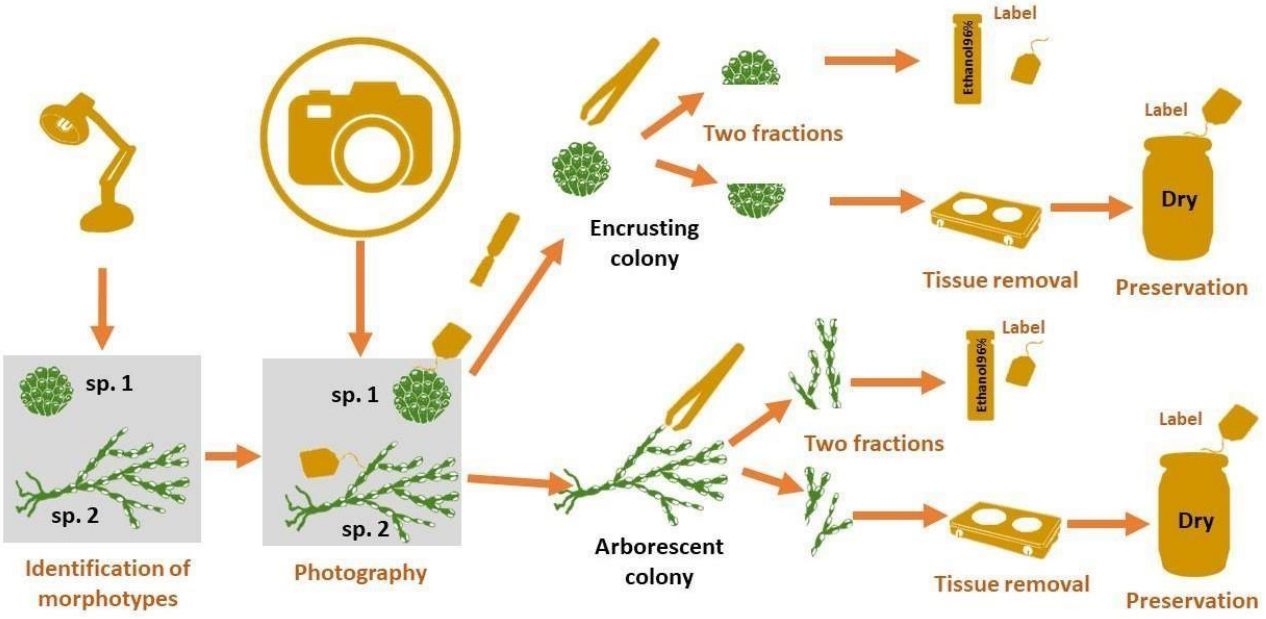


Fig. 10 Sequence of procedures for the preservation of bryozoans from encrusting and arborescent colonies.

Class Hydrozoa

It is necessary to relax each specimen by gradually incorporating drops of 70% ethanol in a container of seawater over a time period of 2 to 3 minutes, until immobility of the zooids is achieved; an alternative is to store at 4 ° C each sample for 2 to 3 hours. After the relaxation process, each colony is divided into two fractions and they are directly preserved in 4% formaldehyde and 96% ethanol (**Fig. 11**).

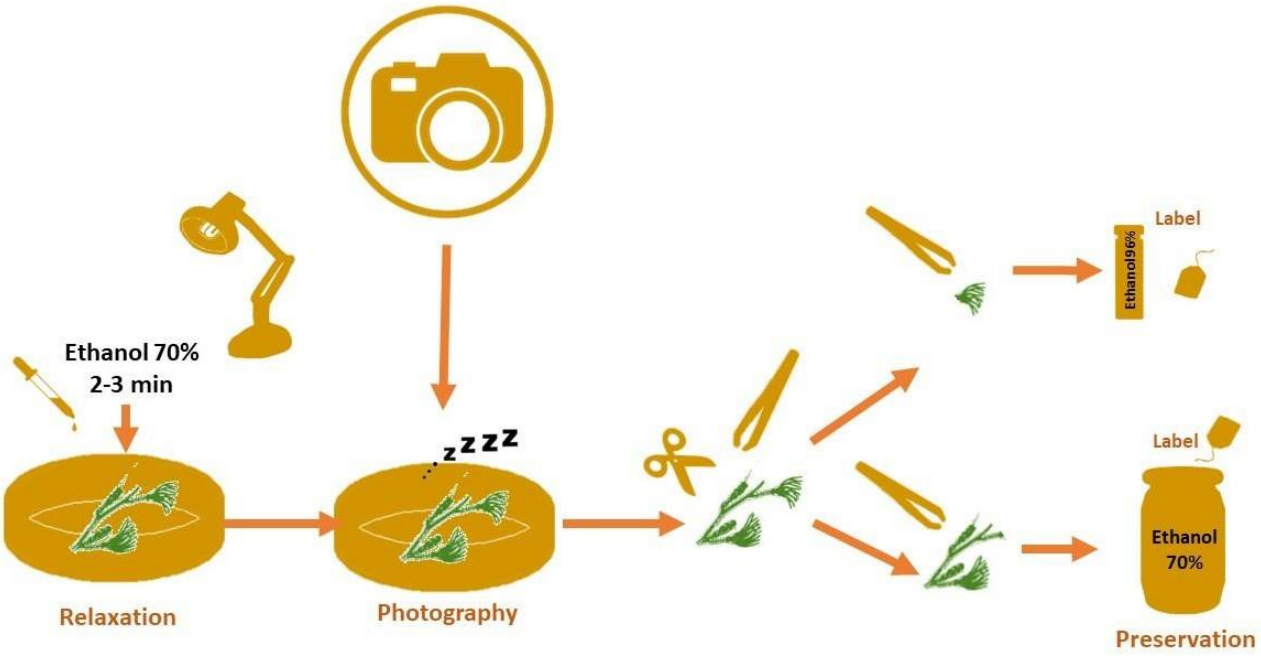


Fig. 11 Processing for relaxation and preservation of hydrozoa. The use of lamps is recommended for observation and discrimination between species.

Class Anthozoa

For corals, the organisms must be carefully separated from the plates with a gouge. If extra pressure is required, a hammer and the gouges are carefully used to avoid damaging the skeleton. To carry out molecular and histology analyzes, tissue samples aretaken; one part is rinsed with running water and stored in containers with 96% ethanol. The other part is decalcified with daily changes of Bouin solution for 3 days and finally fixed in formaldehyde 4%. For the observation of hard structures, it is necessary to remove the tissue, each sample is placed in 10% sodium hypochlorite (commercialchlorine) and/or 0.5% boric acid for 7 to 8 hours. Subsequently, each sample is dried in an oven at 120ºC for two days (**Fig. 12**).

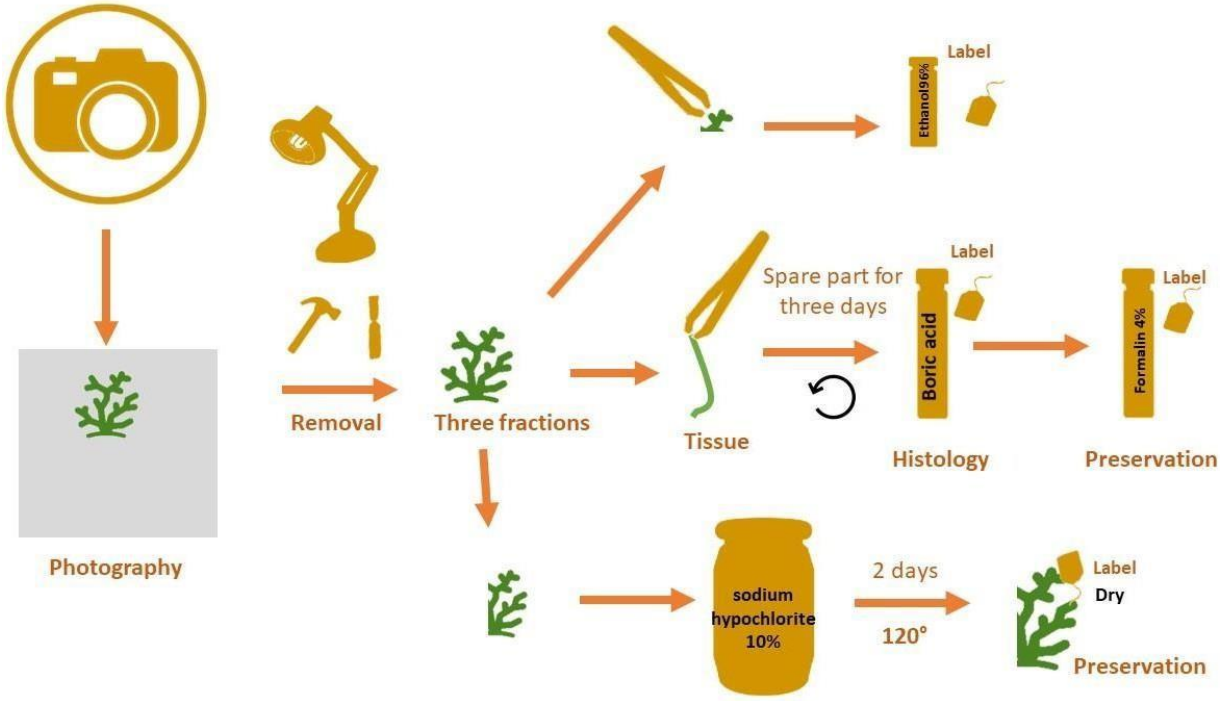


Fig. 12 Processing sequence for sampling and tissue for coral identification.

In the case of sea anemones, it is important to take photographs of the external anatomy of the specimens in vivo, to record the coloration, measurements (use a scale), shape of the column, oral disc, tentacles, pedal disc, mouth, and any other additional structure that can be seen with the naked eye (spots, warts, or vesicles on the column, etc.). Subsequently, the anemones must be carefully separated from the plates with the help of a gouge to loosen the anemones from their base or pedal disk. After that, the specimens must be relaxed with magnesium chloride or 3-4 drops of menthol dissolved in seawater until the organism does not respond to tactile stimulus.

Before fixing the specimens, two tissue samples (approximately 3 mm) should be taken, preferably from pedal disc or tentacles, and placed in microtubes with 96% ethanol and preserved at 4° C. The rest of the specimen must be fixed with 7-10% formaldehyde for at least 2 months; then, it must be transferred to 70% ethanol for preservation (**Fig. 13**).

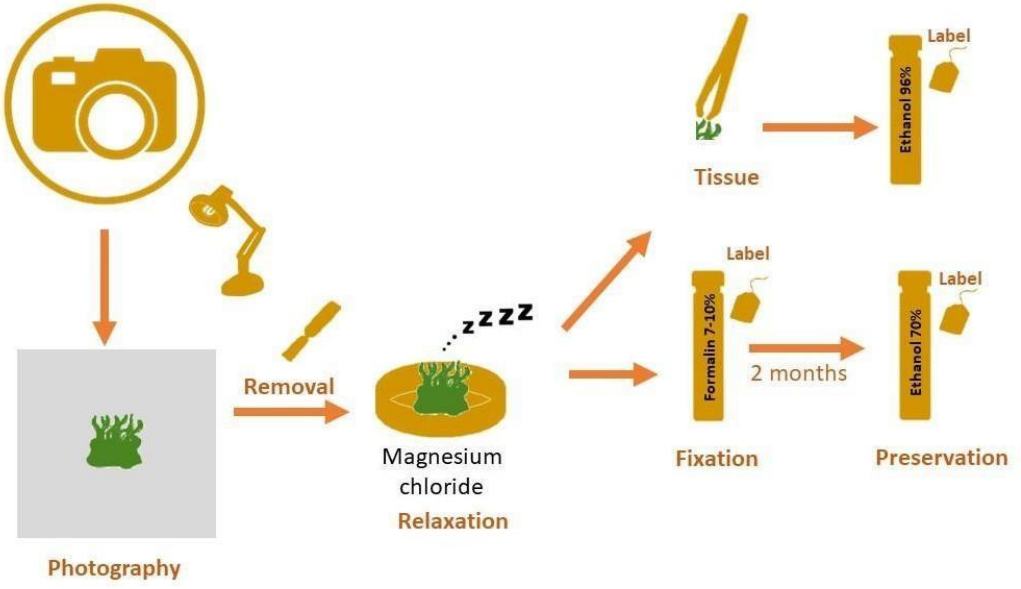


Fig. 13 Processing for the preservation of anemones. The use of lamps is recommended for the observation and discrimination between species.

Phylum Porifera

For sponge specimens, it is necessary to identify morphotypes in the plates, it is also recommended to take photographs of each morphotype (a label number is assigned per each morphotype). Take care of morphological characteristics such as color and type of the surface both are important for the taxonomic identification of sponge specimens. A gouge or scalpel could be used to remove the specimens from the plate. Preserve the specimens directly in crystal jars with filled with 96% ethanol (**Fig. 14**).

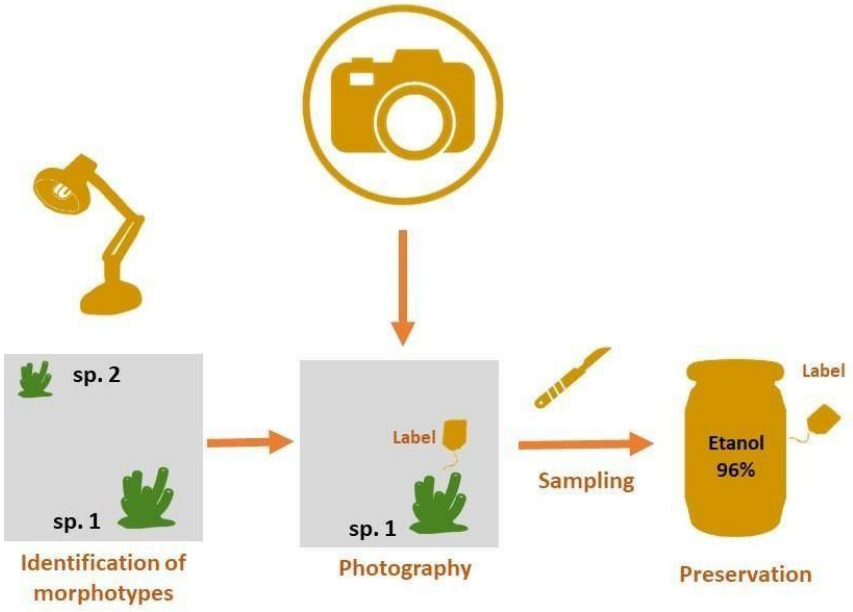


Fig. 14 Processing sponges on recruitment plates.

Phylum Chordata

Tunicata subphylum
Ascidiacea class

It is necessary to identify all the different morphotypes of sea squirts present in the plates and their variations in coloration, as well as to take photographs to help in taxonomic identification (in the case of colonial organisms, pay special attention to the organization of zooids and color patterns, it is also important to keep the information of each specimen: plate number, orientation, and coloration).

After taking pictures, all specimens must be anesthetized. For this process, place a couple of drops of the menthol saturated solution directly on each specimen, and let the solution settle for 15 to 20 minutes with a tray on top). Once the specimens are completely anesthetized (verify there is no response to tactile stimulus, for example, no siphon retraction), proceed with the removal of the animals using gouges. For colonial and solitary specimens, a fraction of each specimen (or colony) is preserved in containers with 4% formaldehyde and another small fractions (few colony zooids and/or a small piece of atrial siphon in the case of solitary organisms) is preserved in microtubes with 96% ethanol for molecular studies (**Fig. 15**).

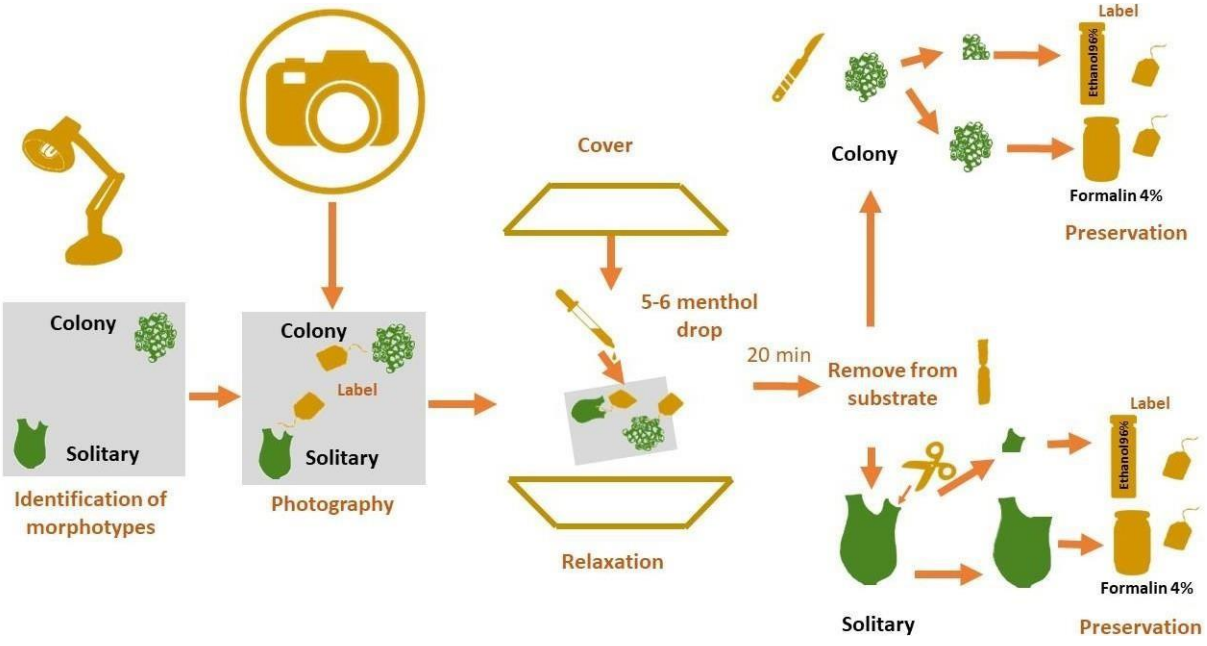


Fig. 15 Processing sequence for relaxation and preservation of colonial and solitary ascidians.

Subphylum Vertebrata

In the case of fish, it is necessary to relax the specimens by adding gradually and continuously 10% clove oil diluted in 70% ethanol. The photographs should be done with the head pointing to the left side for easy observation of taxonomic structures that facilitate their identification. The collection of samples for molecular analysis is performed by the extraction of muscle tissue from the right side of the body and incorporation with 96% ethanol. Finally, the specimens must be labeled and preserved in 70% ethanol (**Fig. 16**).

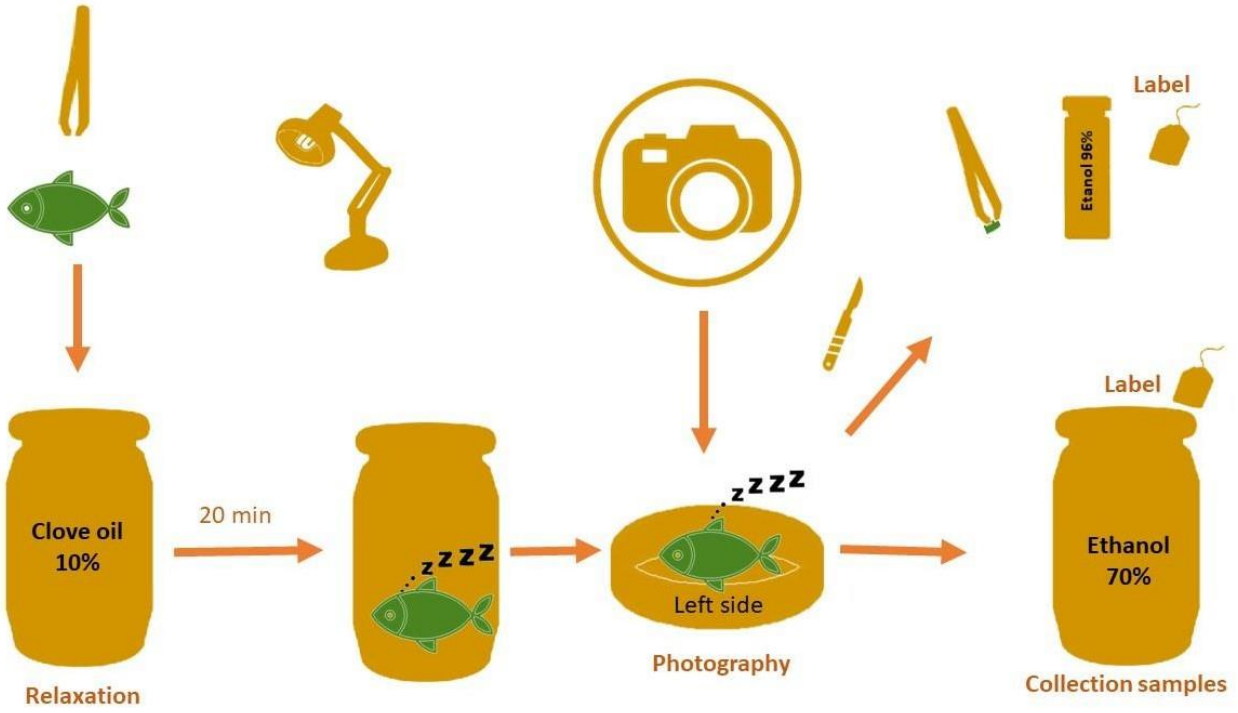


Fig. 16 Processing sequence for fish relaxation and preservation.

Marine fauna associated with ARMS

Date:_____Locality: _____

Code	Plate Orientation	Phylum	Preservation	Color	# of ind. or colonies	General observations

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Mollusks

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Phylum Echinodermata

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Echinoderms

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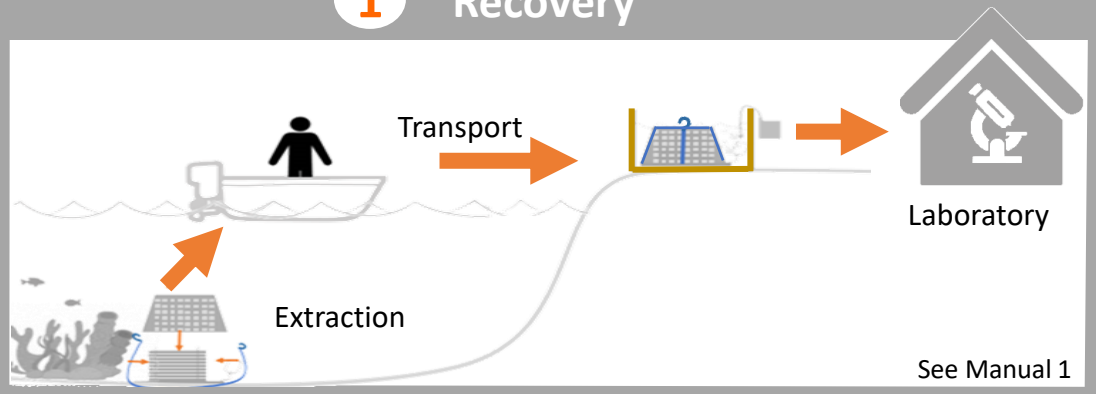
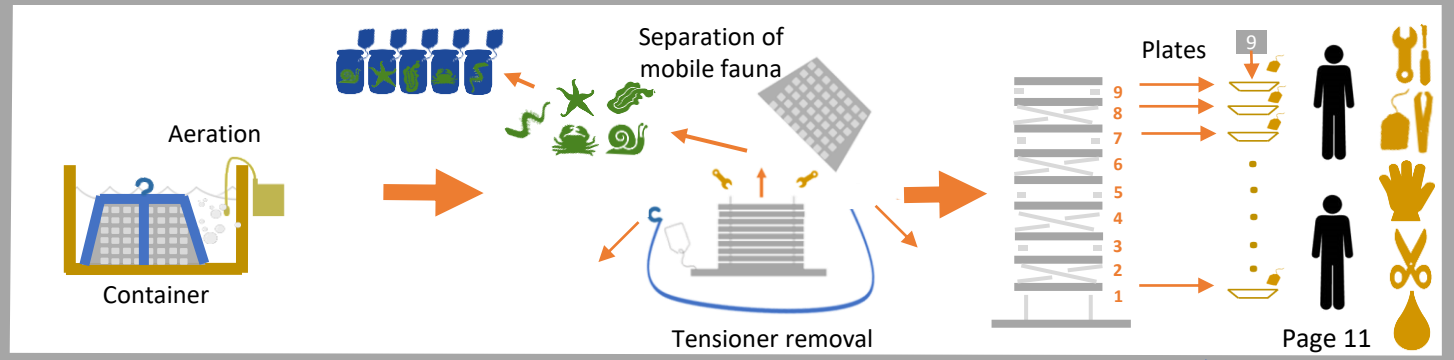
1 Recovery



2 Preparation



3 Fauna processing

TIME

2 hrs | 4 hrs | 6 hrs | 8 hrs

1 Mobile fauna separation

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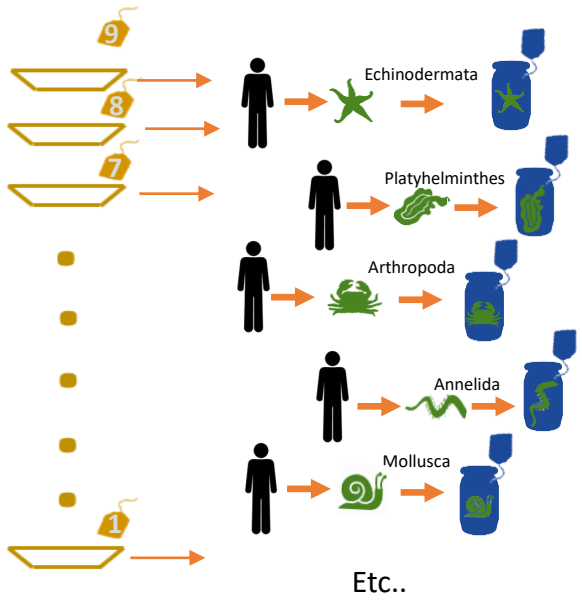

2 Photo shooting

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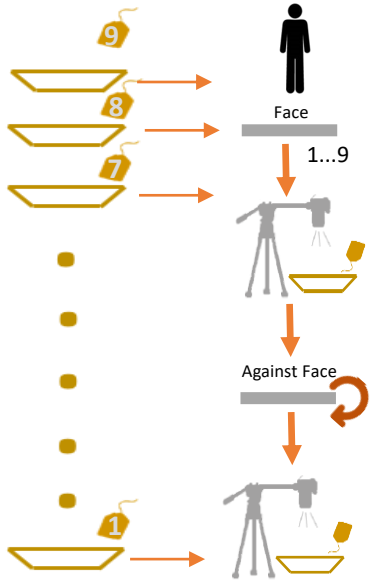
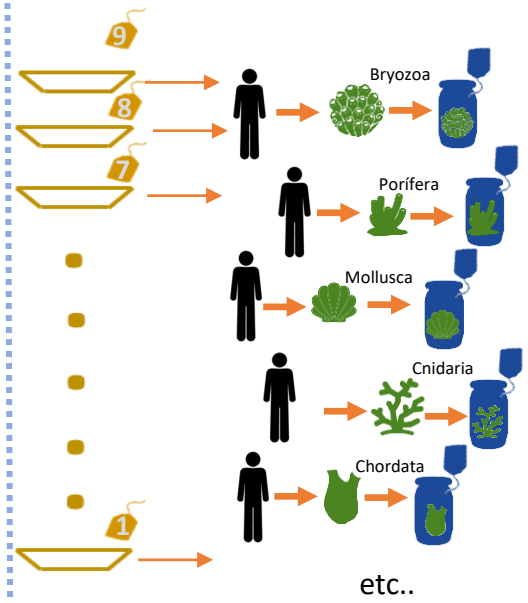
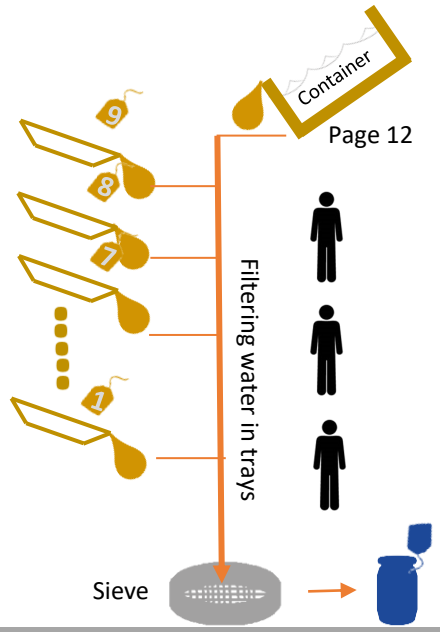


3 Sessile fauna separation

Page 14-29



4 Filtering process



4 Post-processing

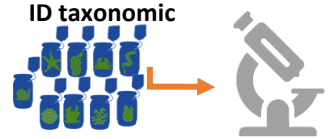
See Manual 3

Photographs

https://hcas.nova.edu
https://coralnet.ucsd.edu/

+

ID taxonomic



DarwinCore

https://dwc.tdwg.org/

Material

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