azti tecnalia

GECKA

Genetic close-kin analysis on white anglerfish (*Lophius piscatorius*) for abundance estimates in support of deep sea fisheries management under the Common Fisheries Policy

SAMPLE COLLECTION MANUAL

PROJECT TITLE: Genetic close-kin analysis on white anglerfish (*Lophius piscatorius*) for abundance estimates in support of deep sea fisheries management under the common fisheries policy

ACRONYM: GECKA

FUNDING BODY: JOINT RESEARCH CENTER, EUROPEAN COMMISION

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1. INTRODUCTION

The European Commission recognizes the need for developing an improved management framework for deep sea fisheries, which are particularly vulnerable to exploitation and for which little scientific knowledge and data are available. Recent research has shown that the genetic close-kin mark recapture (CKMR) method can be employed for obtaining fisheries-independent abundance estimates in marine fish, which would be extremely valuable for deep sea stocks. Yet, the viability of the method needs to be evaluated considering the general lack of biological knowledge for deep sea stocks. In this context, the need of using a better studied species with well-known biology and life-history traits as a target for exploring the viability of the method and required modifications has been suggested. For this reason, although not strictly a deep-sea species, it has been considered that the white anglerfish (*Lophius piscatorius*) could be a good candidate target species for i) being commercially important, so that the development of the method for this species is directly relevant ii) allowing relative easy sample collection, so that a large number of samples from which to subsample could be obtained, and iii) being sufficiently studied, so that minimum biological and reproductive parameters are known. Nonetheless, an essential requisite for the successful application of the CKMR method is the knowledge of the population substructure of the target species. Although previous studies have addressed this question for white anglerfish within the Atlantic and suggest homogeneity, additional support is required. Thus, the main objective of the project is to establish the genetic population structure of the white anglerfish across the Atlantic FAO fishing area 27 and to provide a solid baseline for a close-kin abundance estimate.

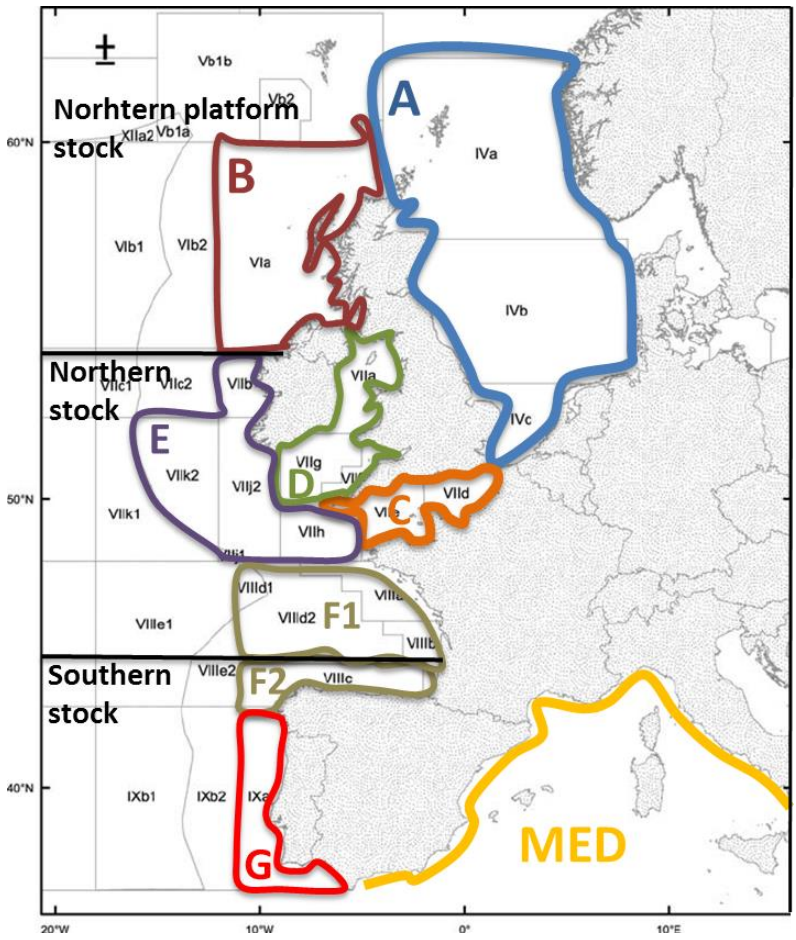


Figure 1: The main sampling areas defined for the project

2. OBJECTIVE

The objective of this protocol is to obtain a collection of samples of white anglerfish (*Lophius piscatorius*) and associated metadata suitable for deciphering the population structure of the species.

3. REQUIREMENTS FOR SAMPLING

We appreciate that you agreed to collect samples for this study. It is important that you follow the sampling instructions in order to ensure good quality samples and accurate biological and geographical information, which is critical for the success of the study. Please, do not alter or guess any measurement as erroneous data can be translated into erroneous conclusions. If a measurement is missing, please leave it blank and explain why.

3.1. Number of fish

We kindly ask you to collect a minimum of 50 and a maximum 100 fish for us per area.

3.2. Criteria for selecting the fish

In order to obtain good quality samples, fish have to be sampled right after finishing the fishing operation. If possible, follow these indications:

- If a wide range of sizes is found, collect fish covering the size range, including smallest and largest
- If specimens from both sexes are found, collect samples from both sexes
- Avoid sampling all fish from the same catch.
- If you are collecting fish from more than one area (from those in Figure 1), take at least 50 from each.

3.3. Facilities for biological sampling

For the benefit of you, but also for achieving the best quality samples, it is important you establish a good base for your biological sampling before going on board of the fishing vessel. Read carefully the entire protocol and contact the scientific staff if you have any doubt. Consider that you will need a space on board of the fishing vessel to carry on the sampling, which should include:

- Sufficient space for you to work comfortably, and sufficient space to store sample boxes and your sampling equipment;

- Sufficient light so you can see what you are doing;
- Ready access to clean water, for washing your knives, scalpels, tweezers, and for washing your hands.

3.4. Material for biological sampling

You will need:

- A box filled with 1.5 or 2ml vials that are prelabeled and filled with ethanol or RNA later
- A scalpel or a sharp knife
- Tweezers
- Gloves
- Ethanol or sterile water for cleaning

We will provide the box with the pre-labelled vials. If you require us to provide any other material, please let us know.

3.5. Sample Sheet for data recording

The Sampling Data Sheet (see Appendix 1) is your main system of recording. For each fish, you need to register:

- ID: a consecutive number from 1 to 100
- Other ID: if the institute collecting the samples has other coding schema for the same sample
- Vessel: name of the vessel collecting the samples
- Fishing gear
- Sampler: your name, so that we can contact you if there are doubts
- Day, month, year
- Latitude and longitude: it can be in decimal or DMS, but please indicate which unit you used
- Depth of the catch
- Length, weight, sex and maturity stage of the fish (see below for details)
- Any relevant note or observation

4. SAMPLING PROCEDURE

The following is the recommended order of steps in your biological sampling. Keeping to this order is important for the standardization of sampling across locations and across sampling teams.

4.1. Confirming species ID

It is critically important that you confirm that the species you are sampling is *Lophius piscatorius.*

4.2. Collect genetic sample

The highest priority in the taking of a sample for the genetics analyses is to ensure there is no cross-contamination between samples and to take the sample as close as possible to the fish death to avoid DNA degradation. Remember to use ethanol for cleaning all instruments (or bottled water if ethanol is not accessible).

The following is the recommended procedure:

- Use globes throughout the process;
- Clean one side of the fish using fresh tissues moistened with clean water to remove any blood and/or mucous on the skin;
- Using a scalpel or a good sharp knife, make a small incision on the cleaned side of the fish;
- Take a slice of tissue (without skin) that is not larger than $1cm^3$ (about the size of your small finger nail) – it is important that the tissue is not bigger than that as, otherwise, ethanol or RNA later will not penetrate, and DNA will be degraded;
- Using tweezers, transfer the muscle tissue sample direct to the pre-labelled vial, making sure that it is totally covered by ethanol or RNA later and ensuring no contamination from your fingers at the mouth of the vial and seal the vial with the lid;
- Pace the tube into the box making sure that the tubes remain in the same order as they were

4.3. Register data

4.3.1. Fish sample ID

It is important that the data is associated to the correct fish. Please note the code registered in the sample vial into the Sample sheet as well as any other ID used for that fish (if it will be used for other purposes at your institution).

4.3.2. Date, location data

Note, sampling vessel, sampler name, date, location (lat, lon), depth and gear for each fish.

4.3.3. Fish length (mm)

Total length (TL: from the tip of the snout to the tip of the longer lobe of the caudal fin, usually measured with the lobes compressed along the midline) should be measured to the 1 centimeter below.

4.3.4. Fish weight (g)

Total weight (TW) should be measured to the 1 gramme below. If only gutted wigth measurement is possible, please annotate that GW was used.

4.3.5. Fish sex

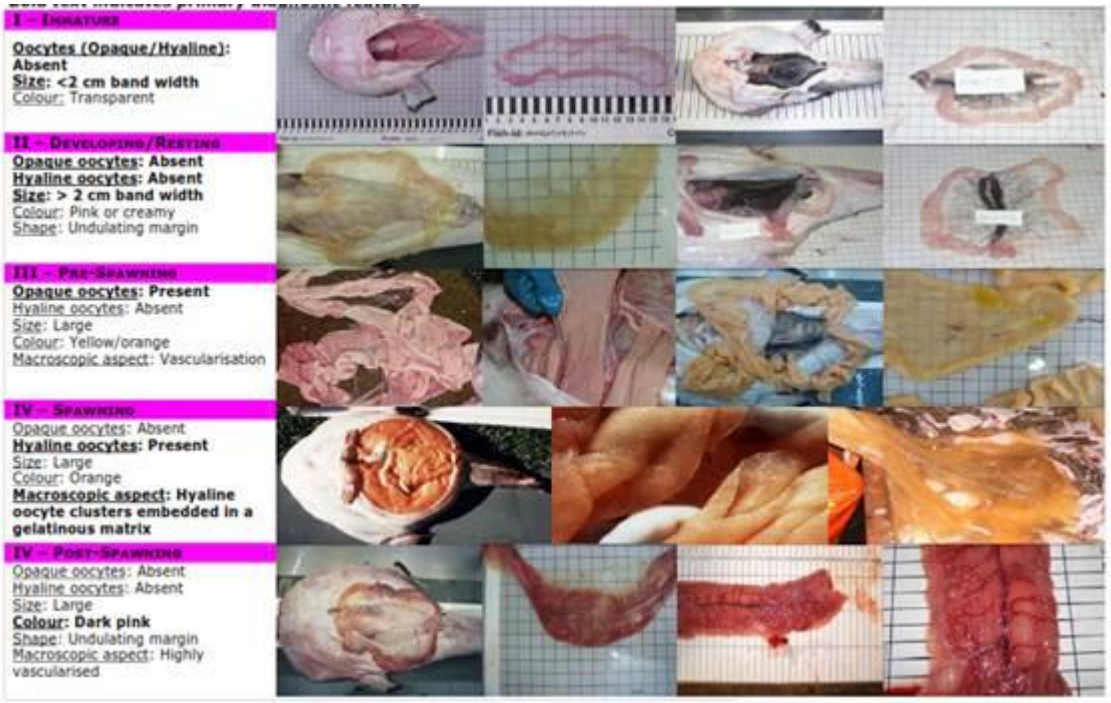
Sex should be annotated as 0 for undetermined, 1 for males and 2 for females.

4.3.6. Fish maturity

Fish macroscopical maturity should be assessed according to the maturity scale given by WKMSHM, 2007 (Figure 2):

1- Immature
2- Developing/Resting
3- Pre-spawning
4- Spawning
5- Post-spawning

Note: mature resting stage has been aggregated along with stage II (Immature) due to difficulties in discriminating both stages. The correct identification of these stages is only possible by means of histology. However, samples in the spawning season should minimize this inaccuracy, when the proportion of resting females should be minimal.



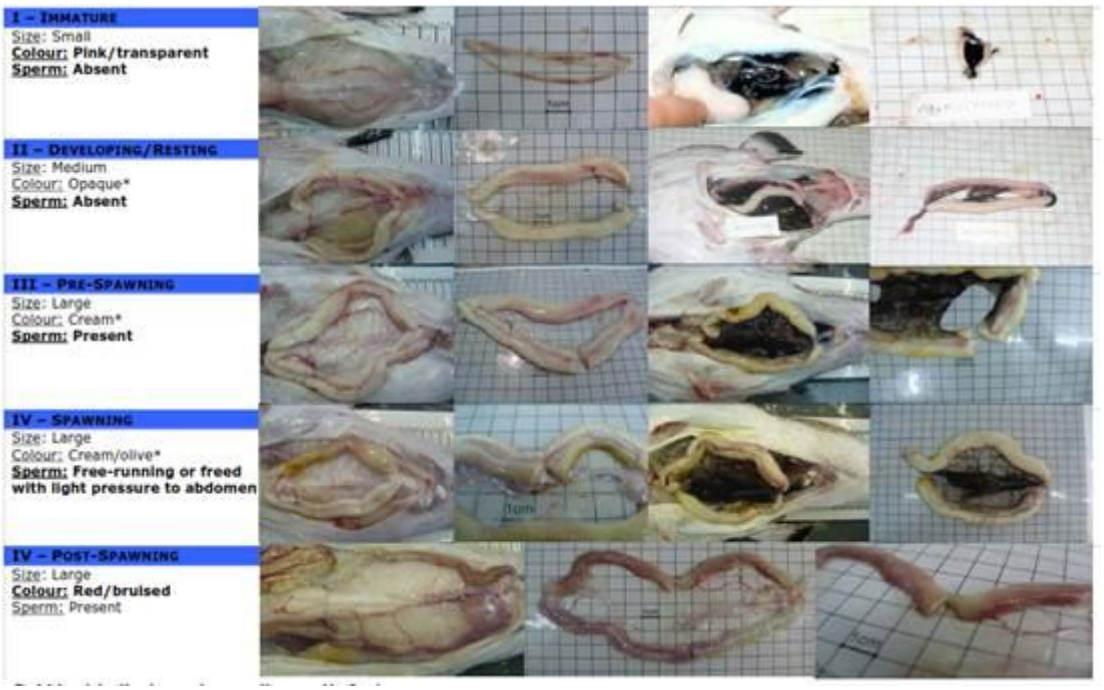


Figure 2. Maturity stages for female (above) and male (below). Note that the last stage should be V and IV [this was an error in the original publication]

4.4. STORAGE, TRANSPORT AND DELIVERY OF SAMPLES

During the fishing trip genetic samples must be stored at the lowest possible temperature. Once in port, all samples should be immediately shipped to the project coordinator or stored at -20 C or below.

Once, you have the samples, please contact the project coordinator (Naiara Rodriguez Ezpeleta: nrodriguez@azti.es) in order to organize the shipping.

Sample recipient address is:

Naiara Rodriguez Ezpeleta / Iñaki Mendibil

Txatxarramendi ugartea z/g

Sukarrieta 48395 Bizkaia

Spain

APPENDIX A

AZTI ID	Other ID	VESSEL	Fishing gear	Sampler	Date	Lon (º)	Lon (')	Lat (º)	Lat (')	Depth	Length	Weight	Sex	Maturity stage	Notes