Protocol A - Sampling and slaughter methods [Pratical guide of protocols: sampling procedures]

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Protocol A
Sampling and slaughter methods

I - Introduction

Sampling procedures are commonly carried out in both laboratories and farms in order to collect samples from animals under experimentation or rearing conditions. In the laboratory, these procedures are necessary to analyse the effects of different treatments (i.e., hormonal, environmental or genetic cues among others) on fish performance under investigation. Like other scientific areas of knowledge, the reproduction area is vast and complex. In particular, the study of those factors involved in the activation of the brain-pituitary-gonad (BPG) axis is crucial for a better understanding of some biological processes related to the estimation of male and female reproductive success in fish. Accordingly, a general overview about practical considerations of how to proceed in a sampling and slaughter procedure to study reproductive aspects of this axis is described in this session. It should be noted that the equipments and protocols available in the laboratory for each species are a key issue to be considered before samples are collected. On the other hand, according to the objectives in each experiment, variations in the way to proceed in sampling and slaughter procedures may occur. A conventional procedure for sample and data collection is described in this Protocol including blood and plasma collection, dissection and tissue collection and estimation of different body indexes in the sea bass.

II - Purpose

The objective of this section is to show a general overview about practical considerations of how to proceed in a sampling and slaughter procedure. It should be noted that different procedures might be used according to the aims of each experiment the required samples. Thus, the description of this Protocol is presented as a conventional sampling, to which some modifications may be necessary.

III - Procedure

(i) Different methods for collecting samples are addressed according to the analysis to be conducted. If internal organs are required, fish must be sacrificed. It is usually carried out on ice. If fish need to be alive, they must be anaesthetized. Induction of anaesthesia can be carried out using MS-222 (0.1 g l^{-1} of seawater), 2-phenoxyethanol (0.5 ml l^{-1} of seawater) or clove oil. The above doses may be lethal after about 20 min, thus fish must be handled gently in order to obtain samples and they must be rapidly returned to the tanks. The use of gloves is recommended during the whole process.

(ii) Weigh and measure the length of each fish.

(iii) Collect blood from the caudal vein in heparinized syringes and keep on ice in 1.5-ml heparinized eppendorf tubes. Later, centrifuge blood at 2.500x*g* for 30 min at 4ºC and collect plasma. Store plasma at -20ºC until analysis using 0.5-1.5-ml eppendorf tubes which must be clearly labelled. Store plasma samples in duplicate, if necessary.

(iv) Excise tissues of interest by an incision through the abdominal cavity just behind the pectoral fins (i.e. a caudo-craneal dissection) using scissors and tweezers. Expose abdominal organs and commence with tissue collection.

(v) Record the individual weights of liver, visceral fat, gonads and carcass for calculation of the hepatosomatic (HSI), fat visceral index (FVI), gonadosomatic index (GSI) and carcass index

(CI), respectively (Table A.1). The liver is readily recognisable due to its typical colour, i.e., pink-brown, although it may vary between species. The fat is also readily recognisable and it shows a typical white colour around the gut. The gonads are readily recognisable in large fish and mainly during the reproductive season. Testes are white in colour while ovaries show a red-orange colour with recognisable eggs. Small fish show very thin gonads, which are usually attached to the swim bladder. They have to be carefully excised using scissors and tweezers. For histological analysis, a small piece of gonad must be rapidly fixed into labeled glass vials containing the fixative solution.

Table A.1. Estimation of some body indexes

Body indexes	Formula
Hepatosomatic index (HSI)	HSI = (liver weight x body weight^{-1}) x 100
Fat visceral index (FVI)	FVI = (fat visceral weight x body weight^{-1}) x 100
Gonadosomatic index (GSI)	GSI = (gonad weight x body weight^{-1}) x 100
Carcass index (CI)	CI = (eviscerated body weight x body weight^{-1}) x 100

(vi) In order to collect brain and pituitary, head truncation must be carried out. If fish are small, scissors and tweezers can be used for the truncation. Once the head has been truncated, cut through the head transversely drawing a triangle. Carefully, expose the brain by slicing off the top of the skull using scissors and tweezers. If fish are large, use a culinary knife for the incision. The brain is readily recognizable due to its white colour and its lobulated shape. The brain must be carefully excised to observe the pituitary, which is a very small white rounded organ on the base of the brain. Tissue samples must be placed immediately in labelled eppendorf tubes and frozen in liquid nitrogen.

(vii) All data must be recorded on a sampling sheet previously designed in each sampling (see example in Table A.2). Note any observation during the sampling procedure and take care to ensure that samples are correctly collected and stored.

IV - Materials and equipment

- Scale
- Ichthyometer
- Eppendorf tubes
- Syringes and needles
- Storage boxes
- Scissors and tweezers
- Refrigerator or fridge (-20ºC or -80ºC)
- Gloves, lab coat and boots
- Sampling sheet

V - Reagents and solutions

- Anaesthetic: MS-222 or 2-phenoxyethanol (0.1 g l^{-1} and 0.5 ml l^{-1} of seawater, respectively). Alternatively, the induction of anaesthesia can be carried out using clove oil. Clove oil has been evaluated as an effective anaesthetic in sea bass and it can be used at almost 10-fold lower doses than 2-phenoxyethanol.

- Heparin

- Ice

- Liquid nitrogen

- Fixative solution

VI - Results and discussion

There are different protocols that can be conducted for sampling and tissue collection depending on research purpose and the type of analyses to be conducted. This Protocol shows an example of a sampling in the sea bass in order to evaluate growth and reproductive performances of the animals during the reproductive season (Fig. A.1 and Table A.2). Nevertheless, it should be noted that an appropriate setting up of the sampling is crucial for the success of this procedure.

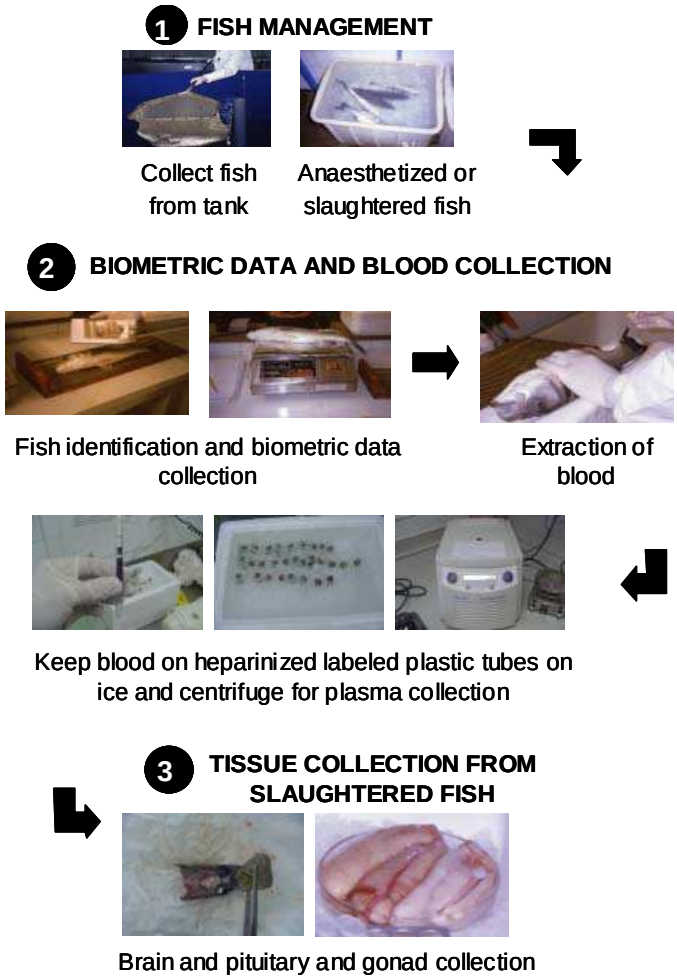

Fig. A.1. Schematic diagram of a fish sampling. (Photographs kindly supplied by V. Cerqueira and A. Felip)

Accordingly, previously to sampling, some aspects need be considered. They include an appropriate labeling of the tubes for tissue collection, the preparation of material and reagents necessary for the sampling, the design of a record sheet to collect data and the distribution of tasks according to the amount of people involved in the sampling.

Table A.2. A sample record sheet for tissue collection and biometric indexes

Date:
Tank: Experiment:
Operator:

Nº fish	Weight (g)	Length (cm)	Liver (g)	Fat (g)	Gonads (g)	Carcass (g)	Sex
1							
2							
3							
4							
5							
...							

Observations: