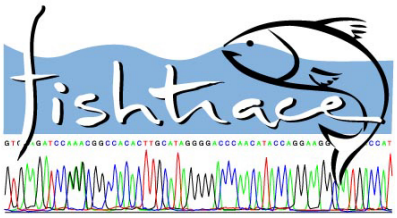
FISHTRACE WP2 PROTOCOLS AND PROCEDURES



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Sven O Kullander and Michael Norén
Department of Vertebrate Zoology
Swedish Museum of Natural History
POB 50007
SE-104 05 Stockholm
SWEDEN
E-mail sven.Kullander@nrm.se, michael.noren@nrm.se

The tasks of WP2 activities are:

1. Collect specimens
2. Collect regional data on the specimens, including common names, field marks, biology, size, fisheries, and forms of use, transformation, etc.
3. Identify species
4. Sample muscle tissue and otoliths
5. Transfer tissue to molecular labs and otoliths to collections
6. Incorporate specimens in biological collections
7. Evaluate taxonomy of each species
8. A regional technical list of relevant publications on taxonomy, distribution, ecology and biological parameters
9. Submit all data to FishTrace database

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Fish sampling protocol

Sampling site
Fish specimens may be obtained fresh in markets or by active fishing efforts.

Field sampling protocol
A protocol for field data recording is available and should be used. The protocols are archived by each partner. Copies of field protocols must accompany specimens/tissue samples/otoliths sent to reference collections/molecular laboratories.

Specimen numbers
For each geographic area is required at least 5 specimens of each target species as specified in the list of target taxa. For haplotype analysis, performed on certain taxa, a total of 15 specimens are required for each geographic area (5+15).

Specimen treatment
The five specimens should be processed minimally as follows:

Specimen #1: Photograph
Muscle tissue: 2/3 samples, one for analysis, one for backup, one for reference collection
Voucher: send whole specimen to reference collection

Specimen #2: Photograph
Muscle tissue: 2/3 samples, one for analysis, one for reference collection, one for backup
Otoliths: extract both sagittal otoliths, photograph right one and send to reference collection
Voucher: send whole specimen to reference collection

Specimen #3: Photograph
Otoliths: extract both sagittal otoliths, and send to reference collection
Muscle tissue: 1/2 sample, keep it preserved
Voucher: send whole specimen to reference collection (study specimen)

Specimen #4: Photograph
Muscle tissue: 2 samples, one for analysis, one for backup
Voucher: send whole specimen to reference collection = NRM

Specimen #5: Photograph
Muscle tissue: 2 samples, one for analysis, one for backup
Voucher: send whole specimen to reference collection = MNHN

Haplotyping: Specimens #6-20: Take muscle tissue; dispose of specimen as preferred

Specimen	**1**	**2**	**3**	**4**	**5**
Photograph	**+**	**+**	**+**	**+**	**+**
Tissue Analysis	**+**	**+**	-	**+**	**+**
Tissue Backup	**+**	**+**	**+**	**+**	**+**
Tissue Voucher	**+**	**+**	-	-	-
Otolith	-	**+**	**+**	-	-
Otolith photo	-	**+**	-	-	-
Voucher	**+**	**+**	**+**	**+ (NRM)**	**+ (MNHN)**

Specimen quality
Specimens should be sampled fresh as far as possible. Frozen specimens or specimens that have been on ice for several days are less suitable for molecular analysis. Specimens may be whole or gutted, but intact specimens are preferred. All material must be collected for this project, and old specimens cannot be used.

Specimen size
For tissue and otolith sampling, as well as for systematic analysis, adult specimens showing diagnostic marks are always preferable. For some taxa, it may be more realistic to sample young specimens, or to use adults as primary tissue source, and juveniles as backups.

Special conditions for large specimens
When large size, collection space limitation, or fishing/conservation restrictions make it expensive or difficult to preserve available specimens, e.g., of marlins or swordfish, one of three strategies is possible:

1. Take a photograph and basic measurements of the specimen, and 3 tissue samples; release the fish/return to fisherman

2. Take a photograph and basic measurements of the specimen, and 3 tissue samples; keep the head (and tail if possible) as voucher, and extract the otoliths from the head

3. Inquire with the major museums (NRM and MNHN principally), whether they may consider the transportation worthwhile, and if so, perform the usual photography and tissue and otolith sampling. The recipient will then pay the fish and the transport.

Photography
All specimens of a species must be photographed. Additional specimens may be photographed as well, to cover sexes, reproductive status and ontogenetic stages displaying the variability within a species. Specimens photographed should be as fresh as possible

Photos must be digital color images, at a resolution of 1024X768 or more. Photos must be saved in minimally compressed jpeg format (Resolution 'Fine') or in TIFF or similar non-destructive format.

Photographs must be taken of the **left** side of the specimen, except certain flatfishes. In flatfishes, the eyed side should be shown, with the gill opening down. Photographs should be taken in lateral aspect, and when relevant of other aspects (flatfishes: right and left side; anglerfishes: dorsal and ventral aspect). Fins should be spread as much as possible using alcohol-soaked cotton swabs and supports when relevant.

Photographs should be taken in ambient outdoor light, in shadow, and without flash. The background should be a neutral grey. In the case of uneven light conditions, e.g., in early morning or evening sun, the fish should be directed so that more light reaches the dorsum than the ventral parts.

The photo should show:

1. Fish plus a label showing the FishTrace specimen number and a ruler

Image files must be labeled with the specimens fishtrace code, plus the appropriate file extension, e.g.,

MulSur-SB-05.jpg

Specimen tagging
All specimens should be tagged with numbered tags and the tag number indicated on the tissue sample and otolith tubes. The tag number may differ from the FishTrace specimen number. Tagging should be done before photography and sampling. Floy tags should be inserted through the lower jaw, between the dentaries, so that the locking tip is inside the mouth, and the colored tag can be hidden underneath the gill cover.

Floy Tags, and the gun required for insertion, can be ordered from Floy Tags, Seattle. Write to Betsy Conrad betsy@floytag.com for Price List and Catalog. The FD-94 is adequate for general purpose tagging.

NRM uses FD-94 tags, color WHITE, black text with sequential numbering: NRM T 12345 (The T is there to distinguish from other NRM numbers). The Pistol Grip gun is adequate for most users, be sure to add an extra needle.

Each specimen should also be assigned a unique Fishtrace code. The Fishtrace code is constructed from the first three letters of the generic name, the first three letters of the specific name, two letters denoting geographical area, two digits denoting the specimen number. Don't worry – it sounds more complicated than it is!

Species with identical first three letters in generic name and species epithet require ad hoc code, constructed based on the first differing letter.

Example: the second specimen of *Mullus surmuletus* collected in the western Mediterranean will get the following code:

MulSur-WM-02

The geographical codes are as follows:
SB Skagerrak and Baltic Sea
NS North Sea
CB English Channel and Bay of Biscay: CB
CS Cantabric Sea, NW Iberian Peninsula and Azores
MA Madeiran archipelago
CI Canary Islands
WM Western Mediterranean and Bay of Cadiz
EM Eastern Mediterranean (Greek Seas)
EE Extra-European species

Tissue sampling
Tissue sampling should be done on fresh or fresh frozen fish/fish on ice. When handling the fish use disposable latex gloves/equivalent. Clean equipment and change scalpel blades between each specimen.

You minimally need:
Disposable clean gloves
Tissue sample tubes
Scalpel
Tweezers
Marking pen
95% pure ethanol (not denatured!)

Tissue should be removed from muscle right behind the **right** pectoral fin base (muscle from inside the head in case of large fish). Scrape away the scales, and cut a square (about 5 X 5 mm if possible). Tear or cut the sample into smaller pieces before placing in tissue sample tube, and add alcohol. Mark the tube with FishTrace code, and, if more than one sample is taken from the same specimen, **a**, **b** etc. Store sample tubes in a cool place, e.g., a fridge.

a = sequencing
b = reference collection
c = backup

'Permanent' ink markers are not reliable. It is advisable to secure ink text with adhesive transparent document tape, and to mark both the tube and the lid of the tube. It is advisable to maintain tubes in sequential order in paper/plastic boxes.

Chlorine may be used to clean instruments from DNA, but needs careful washing in ethanol.

Tissue sampling for haplotyping. About the same procedure. Vouchers beyond specimen 5 not required to be saved.

Preservation of voucher specimens
You need:
Formalin
Disposable gloves
Injection needle
Plastic glasses for protecting eyes
A plastic tray with tight lid
Nalgene bottles, 1000 ml, 2000 ml, and 4000 ml.

Fish for collections must be fixed in 10% formalin, i.e., 1 part commercial formalin (36-40% formaldehyde in solution) to 9 parts water. Water must only be fresh water, preferably distilled or deionized water.

If possible, use a flat, wide tray with tight lid for initial fixation. If you use round containers, e.g., Nalgene bottles, keep them lying on the side during fixation.

This is the procedure starting with fresh fish

1. Inject a small amount of 10% formalin in the abdominal cavity and in selected thicker muscular parts of the fish. If fish larger than 30 cm, also cut the right side abdominal wall, ca 5-10 cm, to promote entry of fixation fluid. If fish smaller than 10 cm, no injection is needed except if a herbivorous fish.

2. Dip a cotton or cloth swab in 10% or full strength formalin, and pad the fin base with one hand while raising each fin with the other hand, to permanently keep the fins erect. Leave so for a few minutes, but keep the whole fish wet/damp during the process.

3. Place the fish in the fixation container with 10 % formalin. Keep for at least 1 week, maximum 1 month. Ensure several times that the volume of formalin of the fish is present.

4. (Optional; you may send fish to Biological Collection while still in formalin). Rinse the fish in water for a few hours. Run in graded series of ethanol, 25-40-70%, 1 day in each.

With frozen fish, let it thaw thoroughly before fixation.

Otoliths
Otoliths can be removed before or after fixation of a specimen.

Both otoliths (right side and left side) must be obtained from two specimens.

The operation must usually be performed under a stereo dissection microscope with low magnification.

Otoliths are contained in the prootic bulla and more or less visible from the outside of the cranium in many fish species. To extract, lift the gill cover, poke the bulla with the scalpel tip, and cut a round hole on the bulla, extract the sagitta with a fine-tipped tweezer. Avoid scraping the sagitta.

Otoliths from both, preserved and fresh, fish should be washed lightly in water and blotted dry. With preserved fish it is important to remove all traces of formalin by more intense rinsing in water.

Otoliths should be kept individually and dry in small tubes, with an inner label stating the FishTrace specimen number, the tag number (optional), and from which side each sagitta was extracted (left or right).

Otolith image file name should be FishTrace number plus Otolith:

MulSur-SB-04-otolith.jpg

Identification
Specimens should be identified to species in the field using standard literature references.

Species names should follow FishBase even when known to be incorrect (e.g., *Scophthalmus maximus* instead of *Psetta maxima*). Subspecies names should not be used.

One may extract field guides and PDA pages from FishBase to be used as field reference literature.

Decisions on nomenclature and systematics
The WP2 group will decide on the final scientific name and systematic position of each species as to be shown on the FishTrace website. The decision will be made by consensus agreement and cleared through consideration of a most recent systematic revision and the International Code of Zoological Nomenclature. Decisions will be brought before the consortium for comment before finalized.

Field Morphometric data
Field morphometric information may include the following when applicable, and should be taken from each of the five required specimens:

1. Fresh Standard Length (abbreviated SL). From the tip of the snout to the end of the hypural fan. To mm precision
2. Fresh Total Length (abbreviated TL). From the tip of the snout to the end of the caudal fin (if forked, lobes should be pressed against each other, and the length taken to the tip of the longer lobe). To mm precision
3. Fresh Fork Length (abbreviated FL). From the tip of the snout to the end of the middle caudal fin rays. To mm precision
4. Fresh weight in grammes. Note if gutted or intact

Laboratory morphometric data
Morphometric information taken in the lab may include the following when applicable, and should be taken from each specimen preserved for analysis. The data may be recorded by the recipient collection.

1. Standard Length (as above). To nearest 1/10 mm in fish smaller than 100 mm, otherwise to full mm
2. Head length. From the tip of the snout to the most distant point of the margin of the gill cover (operculum or subopercular as the case may be). To nearest 1/10 mm
3. Body depth. Depends on systematic group. Usually from the ventral/pelvic fin base to the dorsal midline. To nearest 1/10 mm
4. Dorsal fin rays. Separate into spinous (I, II, III, etc), unbranched (i, ii, iii, etc), and branched (1, 2, 3, etc), and give separate count for each dorsal fin (usually one, in mugilids and gobies two, in some gadoids, three; occasionally no dorsal fin present).
5. Anal fin rays. As for dorsal fin
6. Gill rakers. Count ceratobranchial gill rakers only
7. Pectoral fin rays. Count
8. Lateral line scales. Count (optional)
9. Sex (when possible)

Note: This information (less Standard Length which is taken as fresh Standard Length) needs to be sampled in the field for large specimens that are not preserved.

Species information protocol

Species data
Regional and/or general information on each species will be collected as follows:

1. Basic morphology with emphasis on species diagnostic characters. This information will be compiled on a per species basis.
2. Biological information relating to habitat, reproduction, feeding, size and geographic distribution. This information will be compiled on a per region basis for each species.
3. Common names. This information will be compiled both from general sources, such as FishBase and FAO, and on a per region basis within FishTrace.
4. Threat status. This information will be compiled on a regional basis.
5. Fisheries information. This information will be compiled on a regional basis.
6. Socioeconomic information. This information will be compiled on a regional basis.
7. Market appearance of products. This information will be compiled on a regional basis.
8. Bibliography.

Basic morphology. This information will be compiled by WP2 participants and entered in a freetext database field, similar to the information in *Fishes of the Northwestern Atlantic and the Mediterranean*.

Biological information. This information will be submitted by regional groups, and should state:

a. habitat
b. depth range
c. migratory behaviour
d. foraging behaviour (prey capture method)
e. aggregation behaviour (schooling, solitary…)
f. sexuality (gonochorist, hermaphrodite, sex change…)
g. spawning period
h. spawning grounds
i. spawning depth
j. size at first maturity
k. spawning type (scatterer, guarder, livebearing)
l. litter size/egg number
m. known average age
n. known maximum age (per sex if possible)
o. main prey
p. known average size in catches
q. known maximum size
r. current commercial size
s. current minimum size (as enforced by local laws)
t. climate zone
u. north latitude limit
v. south latitude limit
w. general distribution area by geographic descriptors

When information is not applicable it should be indicated as *N/A*; when not known, it should be indicated as *unknown*; when not researched it should be indicated as *not researched*; when not yet considered, leave *blank*.

Common names. This information will be submitted by regional groups. The source of the common name has to be referenced. Note that FishBase names are compiled without real quality check (e.g., most Swedish names are dubious).

Threat status. Regional information will be submitted by regional groups, and should state source of information; this will normally be a national Red List. The WP2 group will supply global IUCN threat status.

Fisheries information. This information will be submitted by regional groups and should consider, in a synoptic fashion per species:

a. Type of fisheries
b. Fishing methods
c. Capture period
d. Exploitation level
e. Commercial interest

Socioeconomic information. This information will be submitted by regional groups and should consider, in a synoptic fashion per species:

a. Forms of use (fresh, fozen, salted, dried, dried and salted, warm smoked, cold smoked, macerated, etc.)
b. Transformed product before commercialization (whole, decapitated, fillet, sliced, roe only, fins, etc.)
c. Cooking options (steamed, fried, deep-fried, grilled, raw, etc.)
d. Typical end-consumer (industry, house-hold, subsistence, restaurants)
e. Consumtion site (local, national, exported)
f. Known market substitutions

If possible products should be photographed as well.

Bibliography. WP2 group will select general bibliography. Regional groups should provide local references obtainable from libraries or official sources. Only literature actually seen and consulted should be considered. The format for references will be provided by the Database group.