T2T MONTSERRAT

2017

A GUIDE TO FISHERIES DATA COLLECTION AND BIOLOGICAL SAMPLING

Joint Nature Conservation Committee

FOREWORD

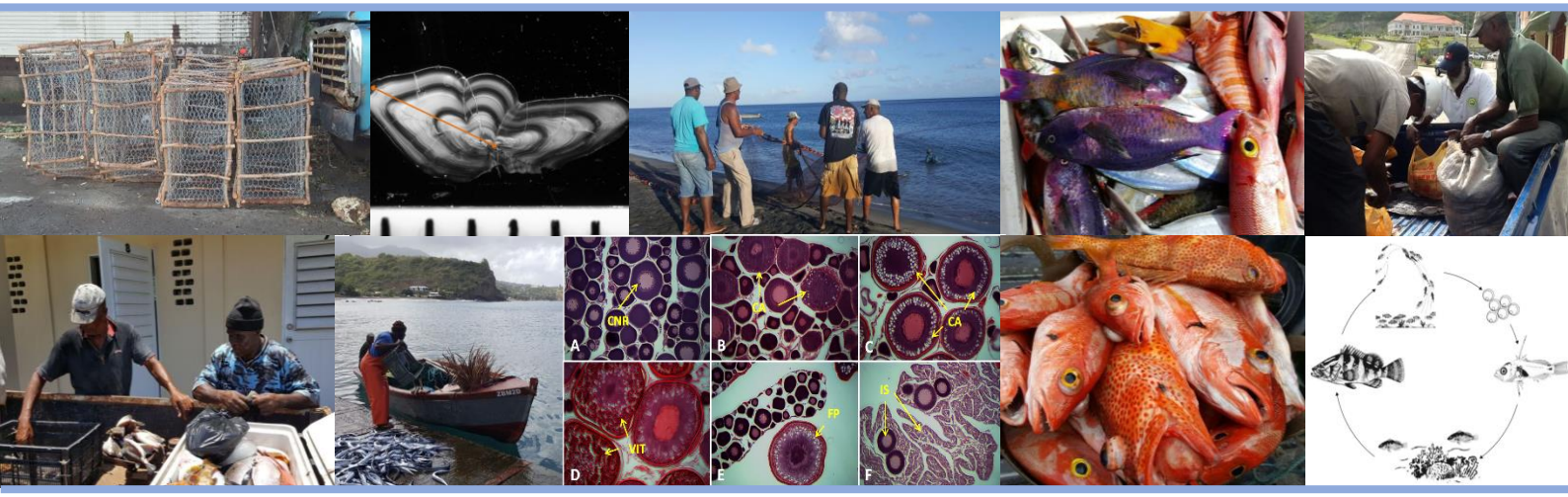
This guide has been developed as an initial aide for data collection in data poor fisheries. The intention is to provide a basic general overview of biological data collection and sampling practices which can be tailored to suit different sampling criteria as needed. For further advice on tailoring this manual for a specific fishery please contact the authors.

No person should undertake biological sampling without first assessing and understanding the risks. Basic risk assessments and COSHH for working with the chemicals and sharp implements outlined which meet national/regional requirements should be produced and utilised accordingly. This manual should not be used alone, without additional training in the methods outlined.

ACKNOWLEDGEMENTS

This manual was developed following the Territory to Territory (T2T) workshop "Collecting quality data to support sustainable fisheries" held on Montserrat, June 2017. The authors would like to thank all participants for their positive feedback and enthusiastic participation in the practical sessions and discussions relating to biological sampling and data collection. It is the authors hope that this manual will prove useful to you all in developing your own sampling programmes.

Photographs courtesy of Alwyn Ponteen and Alice Doyle.



Contributors and Citation

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INTRODUCTION

Data collection is a key component in fish stock assessments. Information from a wealth of sources is used to inform our understanding of fish stocks. This understanding is vital in developing appropriate management tools to support sustainable use of the stock. However, for many fisheries these data are lacking and so management is based on expert assumptions. These assumptions may not accurately reflect the status of the fishery which may lead to stock declines or under-exploitation of an important resource. For data poor fisheries, improving data collection practices is vital first step to improve management.

Sampling programmes provide an excellent route for collecting fisheries and biological data for use by fisheries biologists, managers and stock analysts. It is the authors' intention that this manual act as an aide to biological sampling and data collection in data poor fisheries.

Sampling programme design

In most cases it is impractical to measure all parameters by census (sample the entire stock/catch). Instead, parameters are estimated based on a sub-sample. To be effective, a sampling programme must operate at an appropriate scale which balances resource with data collection that is statistical robust. Sampling frequency is therefore dependant on the resource available and on the size and variability of the fleet. As these factors are fishery dependant, sampling design should be tailored to suit, but should consider the following points;

- ⇨ **Representativeness** is a key factor when designing a sampling programme. This relates to both the boats and the gears they use. There are a few ways to ensure fleet sampling is representative; samples can be a random selection by boat or a random selection by gear type. If using a random selection by boat, prior knowledge of the proportions of the fleet operating each gear type are needed and the accuracy of parameter estimates will be low for certain gears if the number of boats operating those gears are low. Sampling by gear type can give more accurate results but may be more resource intensive.
- ⇨ **Sampling through space and time** is also important. Typically for large fishing areas, the area is split into distinct regions/fishing zones and each zone is sampled. Sampling should be spread throughout the year into distinct sampling periods. These can be monthly, bi-monthly or quarterly. Sampling can occur anytime within a period and can be spread out within a period.

Following these guidelines, a bi-monthly sampling design should sample each fishing zone during each bi-monthly period, and within each fishing zone a random selection of the gears/boats operating should be taken. The number of gears/boats to sample per area per time period will depend on the number of gears/boats in operation. Proportionally, the number of samples needed to get an accurate estimate of a parameter is relatively low but will be influenced by the variability among and between samples. Again, this is fishery specific and will need to reflect the local conditions.

As a minimum, data collected for each sample should include the trip date, vessel ID, gear type, effort (hours fishing relative to gear deployed), fishing area, total catch weight and catch by species (for target species). Each sample should also be given a unique ID known called a trip ID or sample ID. As this guide is intended as a general aide for data collection and biological sampling, target species have not been defined. These will be fishery specific and should initially correspond to those species which contribute most to the overall catch, as well as any species protected under national or international legislation. To ensure that species are not mis-identified due to differences in local names, all target species should be given a code corresponding to the scientific species name. An example target

species table (Table 1) with codes and common names listed is given in Annex 1. Similarly, gear codes should be developed for the common gear metiers used (example also provided in Annex 1; Table 2).

While these fisheries data provide insight on the level of mortality caused by fishing, additional biological measures are required to assess different metrics which influence stock dynamics. Length frequency is used to track fish of a given cohort through time. The addition of aging and maturity assessments enable us to predict the level of resilience to fishing mortality of a given stock.

As these measures are on individual fish the sample pool is much larger. Although sampling for these parameters should still follow similar rules in terms of representativeness and coverage in space and time and this will need to be repeated for each target species, the depth of sampling can be relaxed somewhat to reflect the resources available. Biological sampling could be spread across a quarterly time period. Ideally samples should still be obtained from different fishing areas and gear types, but the numbers sampled can be limited. For length measurements, sampling can be halted once a good length-frequency curve is achieved for each area/gear type. For all other biological measures to be collected regularly, a set number per size class will suffice (e.g. 1-3 weight measurements/ otoliths/sexed and staged animals per cm size class measured; 1-3 gonads sampled per stage identified for each sex) and these can be spread across sampling sessions. It is important to note that if a sub-sample is being taken from a catch for biological sampling then the sub-sample taken should be random (e.g. the first 50 fish, etc...).

Additional samples can be collected on an *ad hoc* basis as resources allow or set projects dictate. Resources should not be wasted taking samples which will not be used.

Data collection Checklist

Real-time data, collected for each sample

- ⇨ Vessel ID
- ⇨ Trip ID
- ⇨ Gear type/fishing method
- ⇨ Effort
- ⇨ Area fished
- ⇨ Date
- ⇨ Catch weight by species for target species
- ⇨ Total catch weight

Real-time data, collected as appropriate

- ⇨ Length
- ⇨ Weight (by individual)
- ⇨ Sex
- ⇨ Otolith collected: yes/no
- ⇨ Gonad collected: yes/no
- ⇨ Other tissue (specify) collected: yes/no
- ⇨ Tissue (specify) weight

Post-sampling data, collected as appropriate

- ⇨ Maturity stage
- ⇨ Age

NOTE:

Although the focus of this document is on data collection from fisheries-dependant sources, data from fisheries-independent sources (i.e. dedicated fisheries surveys) are also crucial to inform fisheries management. Fisheries-dependant data is biased in nature. Fishing gears target specific species and size classes of fish. Fishers themselves are habitual, fishing the same areas year on year. As a result, the data collected on captured fish does not accurately reflect the state of the stock as a whole. To get a more holistic image of the status of a stock, independent surveys should be factored in to the sampling programme. These surveys would be designed to collect samples from stations which are randomly assigned in each fishing area using a non-size selective fishing method. The biological sampling protocols used for these surveys would match those outlined herein.

Additional data collection on environmental parameters (temperature, salinity, plankton communities, etc.) would also help when analysing trends; some of these parameters are simple to ascertain on a regular basis.

General Protocols for Biological Sampling

Following is a brief outline of the basic data collection and biological sampling techniques used to inform fisheries assessments. These should not be put into practice without adequate training in the techniques outlined. Notes on best practice techniques can be found at the end of this section and further details on the techniques outlined can be found in the annexes.

Sampling equipment

- ⇨ Gloves (surgical)
- ⇨ Gloves (heavy duty, puncture resistant)
- ⇨ Data collection sheets (printed on waterproof paper)
- ⇨ Species codes, Gear codes, map of fishing areas (printed on waterproof paper)
- ⇨ Clip-board (for sheets and guides)
- ⇨ Pencils
- ⇨ Marker pens
- ⇨ Measuring board
- ⇨ Tape measure
- ⇨ Heavy duty balance (for catch weight; precision within 1%)
- ⇨ Fish basket (for catch weight)
- ⇨ Precision balance (for individual fish and tissue weights; precision within 1%)
- ⇨ Plastic tray (for individual fish and tissue weights)
- ⇨ Dissection kit
    - Small sharp knife (gutting knife, paring knife or similar)
    - Scalpel handle and large blades
    - Hacksaw/large knife
    - Pointed scissors
    - Blunted scissors
    - Curved forceps
    - Straight pointed forceps
    - Straight blunt forceps
- ⇨ Otolith envelopes
- ⇨ Tissue paper

- ⇨ Sample bags
- ⇨ Eppendorf tubes
- ⇨ Large sample tubes

Fisheries data

Fisheries data can be collected independently, through self-assessment by the fishers (logbooks), and there are also numerous applications available to streamline this process and reduce the data collection burden. However, for the purposes of this manual, it is assumed that dockside/market sampling is the only source for this information and that data collection is taken as a hard copy. The data collection process can then be incorporated or adapted to fit with more advanced data collection techniques.

For all sampling sessions, it is important collect basic fisheries data including the details on the gear used (mesh size, number of lines/pots, etc), the area fished, the length of time spent fishing, the catch weight and composition, the date and the vessel ID. When recording the gear used it is important to note how many lines or pots were used or the length of any nets used. This is important for calculating the effort. In the absence of a more accurate measure, the length of time spent fishing can roughly translate to the time between departure of the vessel and its arrival back to port. However, it is important to be consistent, so if this is the method used it should be used for all trips. If more than one area is fished in a given trip then this should be noted along with an estimate of the time spent in each area.

Any additional data which is thought to be important should also be noted.

Biological data

Catch Weight by species

Catch weight by species refers to the total weight, measured or estimated, for each individual target species. Estimated weights are acceptable where it is impractical to physically weigh all the fish of one species in a given catch. This value is calculated based on the estimated proportion of the catch accounted for by the target species multiplied by the total weight of the catch.

Where possible, the actual catch weight for a target species is obtained by placing all fish in a fish basket, weighing with a heavy-duty balance and subtracting the weight of the empty basket. Catch weight should be measured to the nearest 0.5kg or 1lb.

Measuring Length

There are many ways to measure fish length (see Figure 1). For most species the total length, is the measurement taken, but for some species the fork length may be more appropriate, particularly for species with an exaggerated fork as the delicate fin tissue is prone to damage. Consistency is key; for each target species, you should choose a measure of length and use this for all future sampling.

To get the most accurate measurement length should be taken with a measuring board. The fish should be placed flat on its right side (snout to the left) with the mouth closed and snout pushed against the head of the measuring board. For small species (max size ~30cm), length should be measured to the nearest 0.5cm below; for all other fish, they should be measured to the nearest cm below.

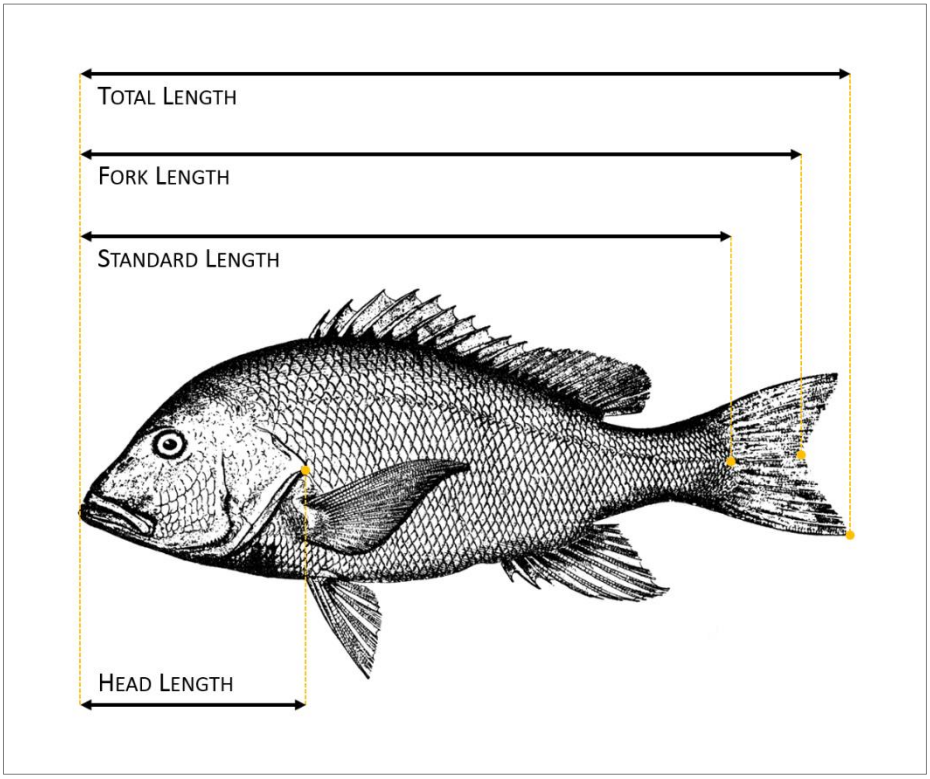

Figure 1. Schematic illustrating the different measurements of length for fish.

Measuring Individual Weight

Weight for individual fish should be measured to the nearest 0.01 kg or 1 oz using a precision balance. Units should always be clear and consistently used. Where possible, whole weight should always be measured. It is possible to back-calculate whole weight from gutted weight where there is enough data to accurately calculate the relationship.

Otolith Sampling

For many species, otoliths can be removed through the gills to avoid damaging the head and subsequently reducing the market value of the fish. To remove otoliths through the gills, lift the operculum and strip away any connective tissue or muscle from the cranium to reveal the hollow chamber in which the sagittal otoliths are contained. Carefully cut through the thin chamber wall and gently remove the otolith using forceps.

For small fish and many pelagic species, it may be difficult to remove the otoliths through the gills. In such instances, an incision through the head just above the eyes can be made to expose the brain. The otoliths can then be found beneath the brain in cavities to the left and right at the back of the cranium. Alternatively, a vertical incision directly behind the eyes can be made, cutting through the otolith chambers, and the otolith can be shaken from the head or plucked out with forceps.

Otoliths should be removed from the same side (all left or all right) where possible. Once removed, otoliths should be cleaned and dried to remove any membrane or mucous and placed in a clean dry Eppendorf tube. The tube is then placed in an envelope marked with the sample's unique identifier, the trip/sample code, the name or code of the species, the data collector's initials and the date. Other details such as the length and sex of the fish can also be included if available.

Removing otoliths whole, with speed and precision takes experience and samplers should take time to practice this skill. Otoliths are delicate and should be handled with care. Working with blades can

be dangerous and precautions should be taken to avoid injury. Fish should always be placed on a non-slip surface when removing otoliths, and puncture-proof gloves should be worn on the weak hand. Depending on the size of the head, cutting tools will vary, ranging from soft pairing knives and scalpels to larger knives and hacksaws; These implements should be kept sharp to avoid using excessive force when making incisions.

Otoliths should not be taken from fish with obvious spine deformities or other abnormalities which could bias the age-size relationship.

For further details on otolith collection, processing and age estimation, see Annex 2

Sex Determination/maturity assessment

For sexually dimorphic species, sex can be determined visually. However, for most species, this can only be determined by examining the gonads, and assessing the developmental stage is *only* possible by examining the gonads.

Fish gonads are usually paired organs of similar size*, located in the abdominal cavity. They are accessed by slicing open the cavity using a sharp knife or scalpel, from the anal pore to the gills. The gonads are usually found at the back of the cavity following the spine (see Figure 2).

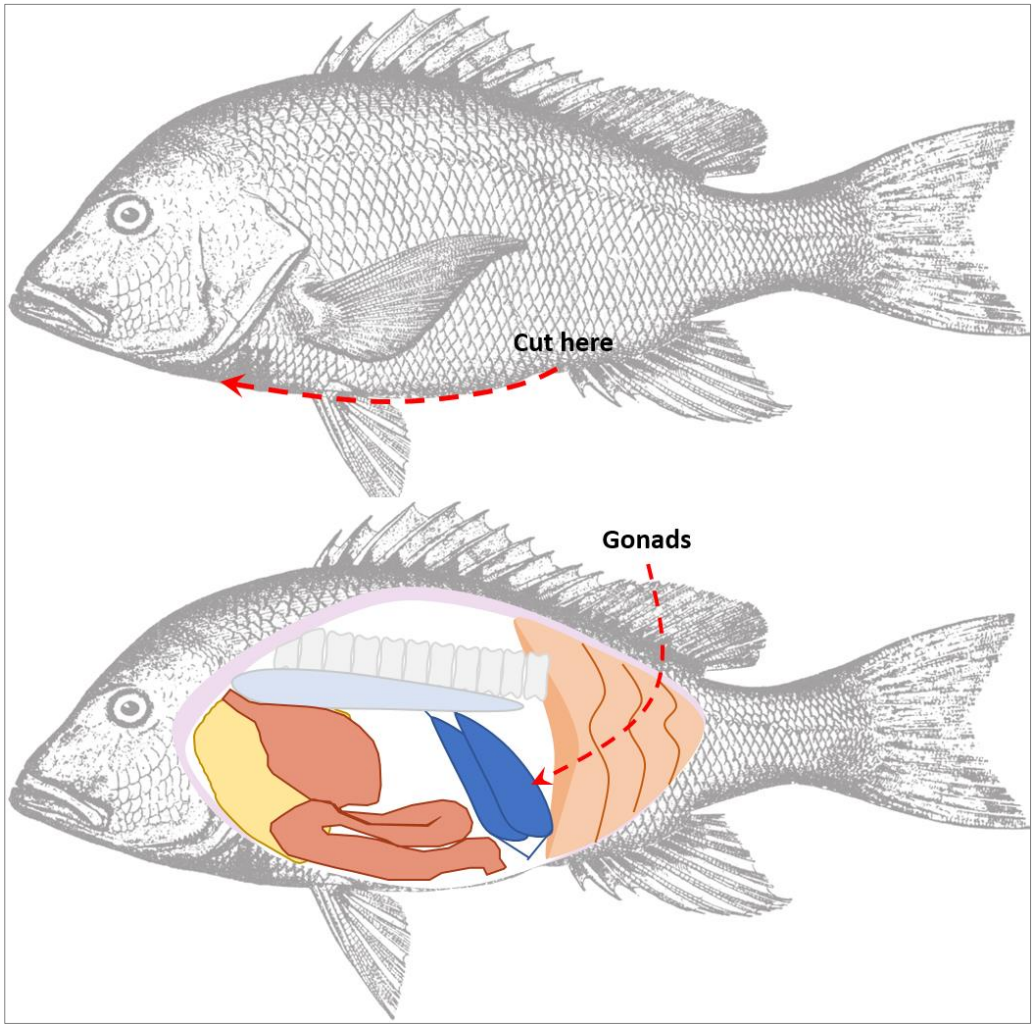

Figure 2. Schematic illustrating the location of the paired gonads in a typical bony fish.

Both the sex of the animal and its maturity stage can be assessed visually using a maturity key as a guide

*For some needlefish species, including the houndfish *Tylosurus crocodilus*, only one lobe of the paired gonads develops as they mature.

Gonad Sampling

Although visual assessment of the maturity stage is useful, it can be difficult to accurately assign maturity stage for certain species. Pelagic species in particular can be difficult to stage. In such cases, histological assessment of maturity stage is essential to obtain an accurate result. This is also a useful methodology to run routinely as a verification of the visual assessment.

Samples collected for histological assessment should not exceed 1 cm^3. For small ovaries, a cross section from the middle of the ovary can be taken. For larger gonads, a sub-sample of a cross section can be taken. The sample is than stored in a vial containing 10% Neutral Buffered Formalin (NBF) at a ratio of 1:10+ sample to solution. The samples can be stored in NBF for some time before they are processed. They should be stored cold in a fridge or cold room.

For further details on gonad staging and histological assessment, see Annex 3

Additional sampling

Additional sampling is dictated by need or interest and should not be prioritised over the data collection protocols outlined above. However, additional sampling can be informative, particularly in data poor regions where little is known of the life histories of species which are targeted by fishing. Some additional sampling protocols are outlined briefly here. These can be expanded upon request.

For **genetic analyses**, ~ 3 gill filaments or 0.5 cm^2 of fin tissue can be removed and stored in vial 100% ethanol at a ratio of 1:10+ of sample to ethanol and stored on ice until they can be transferred to a cold room or fridge. This ethanol should be changed out once the tissues have dehydrated (~48hrs) to ensure the ethanol is not overly diluted, and samples should be stored in a cold room or fridge (preferable). Genetic analysis can be a useful tool for identifying discreet populations and assessing connectivity.

For **proteomic/transcriptomic analyses**, target tissue should be snap frozen over liquid nitrogen vapour and stored immediately at -70°C to avoid degradation of the samples. These analyses are typically used to assess how individual fish interpret and interact with their environment.

For **stomach content analysis**, the stomach and intestines are removed cutting through the oesophagus and the pyloric valve. The stomachs are then immediately frozen and stored at -20°C.

For **stable isotope/fatty acid analyses**, a 1 cm^3 sample of target tissue (typically muscle or liver) should be removed and immediately frozen and stored at -20°C. Stomach content, stable isotope and fatty acid analyses are used to establish diet, to determine trophic level and to identify possible species interactions.

Morphometric analysis is typically accomplished through image analysis. Fish are photographed using a HD camera system which has been calibrated and placed at a set distance from the animal. Measurements are then taken and compared using imaging analysis software. Morphometric analyses can be used to identify morphological differences between sexes or populations which can be a useful, non-invasive tool for field identification.

Measures of **environmental parameters** such as seawater temperature, salinity and plankton communities are very useful for informing trend analyses. Both temperature and salinity can be measured *in situ*. Plankton communities can be assessed through microscopy and will require a water sample to be taken.

NOTES ON DATA COLLECTION

Fish should be identified to species level wherever possible and samplers should be trained in species identification. ID keys for the target species should be taken sampling (it is advised that these are printed on waterproof paper or laminated to prevent water damage and excessive wear and tear).

It is recommended that two people are present when taking biological data – one to measure and one to scribe. However, it is recognised that this is not always possible and lone sampling is often inevitable. It is possible to streamline the process for lone working. For instance, some measuring boards are markable, allowing for quick and easy recording of length frequency by adding a mark for each fish measured for each cm size class. This can then be transferred to the data sheet once all fish are measured. If a board cannot be marked, masking tape can be added along one edge and this can be written on instead. Care must be taken to record different species in differently, either using different marks, different coloured pencils or different parts of the board, and it should be easy to tell which marks are for which species.

If otoliths are being taken alongside length frequencies this can also be streamlined. Otoliths can be taken and placed into an otolith tray in order and the length class the otolith was taken from should be marked “oto1, oto2, oto3...” corresponding to the order. The otoliths can then be cleaned and packaged once all the fish have been measured.

ANNEX 1: SPECIES AND GEAR CODES

Below are species and gear codes adapted from CARIFIS (species) and FAO (gears). These are given as an example of how these might look, but these should be adapted to suit the fishery to which they will be applied. Ideally, these should be compatible with any codes developed by regional or international agencies to which a fishery is expected to report.

Table 1: Species codes

CODE	COMMON NAME	SPECIES NAME
BELO	Gar; Needlefishes	*Belonidae spp.*
SERRGU	Red Hind; Koon; Lucky Grouper	*Epinephelus guttatus*
BALIVE	Queen Triggerfish; Old Wife	*Balistes vetula*
LUTJVI	Silk Snapper	*Lutjanus vivanus*
SPARRH	Bream	*Archosargus rhomboidalis*
ACANCO	Blue Tang; Blue Surgeonfish; Blue Doctorfish	*Acanthurus coeruleus*
LUTJCA	Red Snapper	*Lutjanus campechanus*
ACANCH	Doctorfish	*Acanthurus chirurgus*
CARAHI	Crevalle Jack	*Caranx hippos*
OSTRPO	Honeycomb Cowfish	*Acanthostracion polygonius*
ALBUVU	Bonefish	*Albula vulpes*
LUTJJO	Dog Snapper	*Lutjanus jocu*
CARARU	Bar Jack	*Caranx ruber*
HOLOMA	Longjaw Squirrelfish	*Neoniphon marianus*

Table 2. Gear codes

CODE	GEAR TYPE
FIX	Pots and Traps
LTL	Trolling lines
LX	Hooks and Lines
SB	Beach Seine nets
GEN	Gill nets and Entangling nets

ANNEX 2: AGE ASSESSMENT

Otoliths provide an important insight into the life-history traits of fish. These small structures are formed of calcium carbonate which is laid down in layers throughout the lifetime of a fish. The rate at which the calcium carbonate is deposited varies with the growth of the fish, resulting in the appearance of rings (similar to the rings of a tree) which can be counted to determine age.

Otolith extraction

Otoliths can be extracted from the head in a number of ways – each with varying success dependant on the species. Where fish are typically sold whole, otoliths can be removed through the gills to avoid damage to the head. The otoliths are located in a chamber just below the brain which can be accessed by lifting the operculum (gill cover) and locating this hollow chamber where the sagittal otolith is found. With care, this is cut open and the otolith can then be removed using forceps (Figure 3).

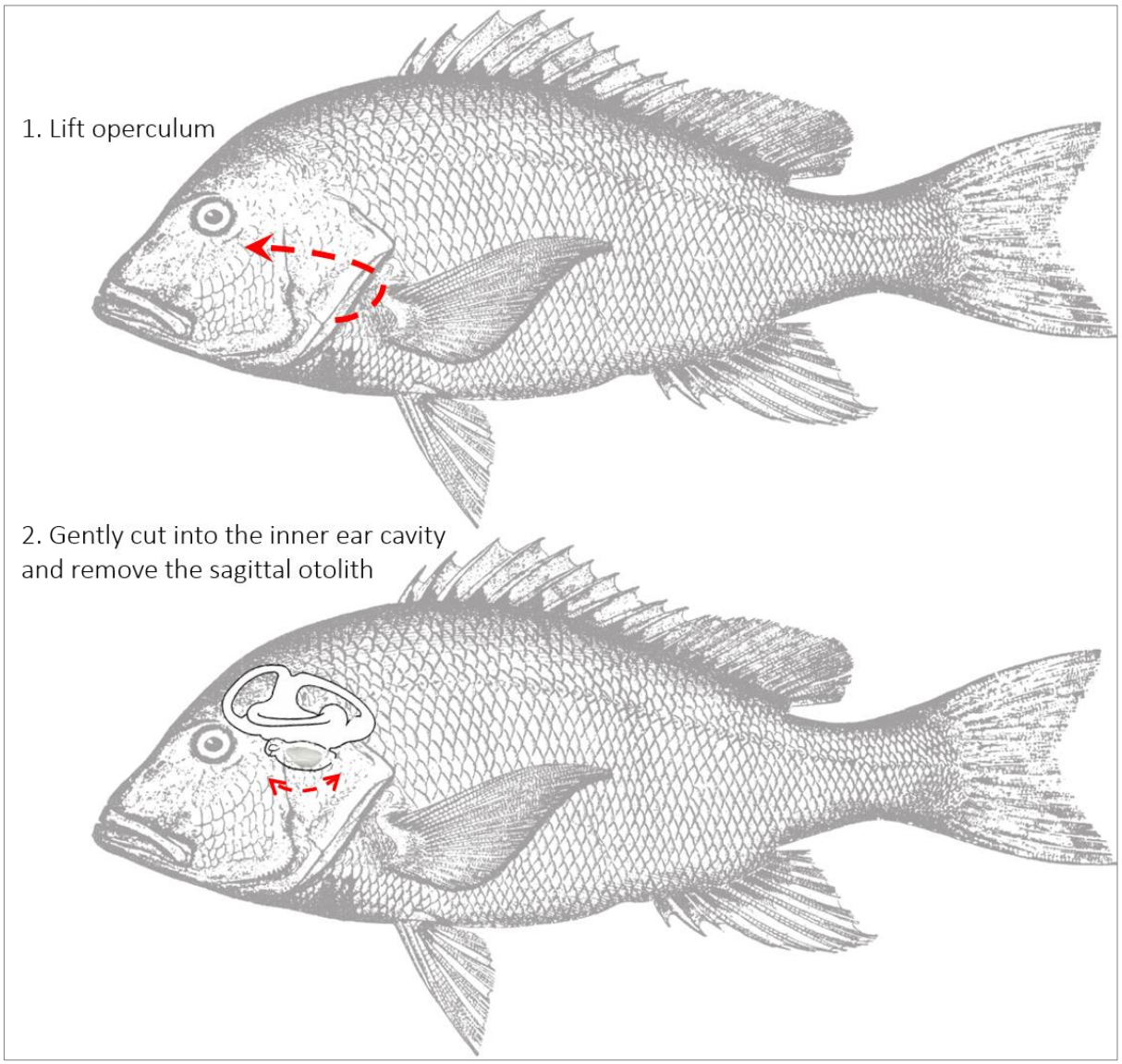

Figure 3. Otolith removal through the gills.

Where extraction through the gills is either impossible or unnecessary, the otoliths can also be obtained by slicing through the head. For fish with large otoliths, the easiest way to do this is to slice through the head in the frontal plane just above the eyes (Figure 4). The otoliths are located in beneath the brain at the back of the skull. For fish with small otoliths (most pelagic species), the head is sliced open behind the eyes in the perpendicular plane, slicing through the otolith chambers (Figure 5). The otoliths can then be shaken onto a clean surface or carefully removed with tweezers.

Otoliths can be quite fragile so care must be taken when removing and handling them. Typically, only one otolith is needed for ageing and the same otolith (left or right) should be removed for each fish were possible. If an otolith is broken then the other otolith can be taken instead. Once otoliths are removed they should be cleaned and dried before placing in an Eppendorf tube and into a labelled envelope with the species name or code, and sample id as well as the sampler's name or initial, the sample/trip code and the date. Other biological measurements such as length, weight, sex and maturity stage can also be included, but these data should be available by cross-referencing against the sample ids.

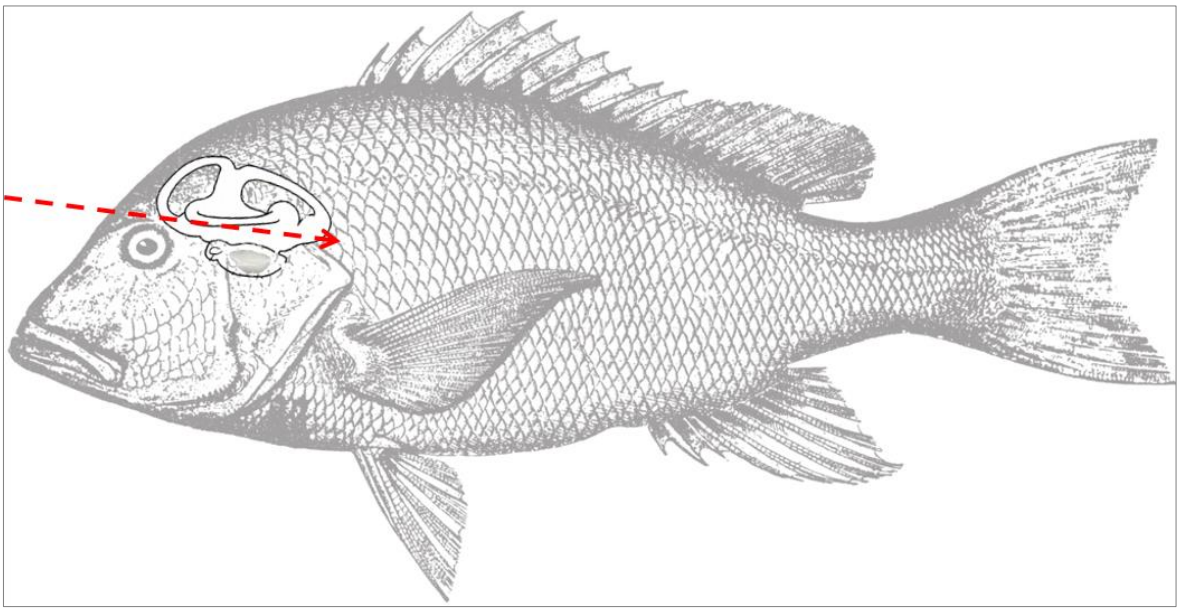

Figure 4. Otolith extraction through the head.

Figure 5. Otolith extraction method used for pelagic species.

Otolith processing

The method described here uses a black polyester resin to embed the otoliths as this provides better contrast when viewing under a light microscope and the resin is flexible allowing for thin sectioning. The marking and alignment steps are important to get right as the otoliths will not be visible once embedded. Other methods often use clear resins which have the advantage of being able to view the otoliths and any labels once the block is set, though these resins are often more brittle and require additional steps to set.

Embedding in resin

Prepared otoliths are embedded in black polyester resin to allow for easy sectioning using otolith moulds developed specifically for this purpose (Figure 6(A)). A layer of resin (50 g per mould) is added to these mounds and allowed to set (~ 12 hrs). Meanwhile, a sheet is prepared to record otoliths IDs and corresponding sample details, their relative positions within the resin block and the unique number of the mould used. To ensure that otoliths are sectioned through their core, moulds are aligned using a mounting jig (Figure 6(B)) with camera system and monitor attached. Two lines marked on the monitor indicate the dimensions of the blade used to cut the otoliths. These marks are aligned to grooves on the otolith mould used to mark the final block, indicating where to cut the final block. There are four rows indicated by these grooves on each otolith mould and each must be aligned individually as otoliths are added. Once aligned, a bead of resin is applied to left hand side of each mould and a piece of spaghetti the same length as the mould is fixed to this to allow for future orientation of the sections and blocks. A bead of resin is then added perpendicular to this along the first row. Otoliths are then added, concave side down, and orientated using the monitor so that the marks indicating the blade width are aligned with the “waist” or “v” of the sulcus (Figure 6(C)). For larger otoliths, ~ ⅓ of the thin end is removed to enhance stability, and otolith position is added to the table after each addition. Otoliths are added in this manner, leaving 2-3 mm between each otolith, for the first row and alignment is double checked before moving to the next row. When all rows are filled, alignment is again checked before these otoliths are allowed to set in place (Figure 6(D)). Once the otoliths are set in place, the moulds are filled with resin (~ 120 g) on a level platform and allowed to harden overnight.

Sectioning

To indicate the alignment of otoliths, resin blocks are marked using the same grooves used to align the otolith moulds with the mounting jig system. A line is etched using a scalpel between these grooves for each row and blocks are marked with a unique identifier as are the rows before the resin blocks are removed from the moulds. Blocks are then sectioned with a mechanical saw using these lines as a guide for the blade. A section of no more than 0.5 mm is cut for each row, and labelled appropriately to allow for cross referencing with the position sheet.

Estimating age from growth increments

Sections are read on a compound microscope using transmitted light. Increments are counted from the nucleus to the terminal edge of the otolith. The state (translucent or opaque) of the terminal edge is determined and the quality of the section and its readability is also assessed. Otoliths with low readability are excluded from further analyses.

Age is estimated based on the number of increments and adjusted to include or exclude the terminal edge based on its composition relative to the time of year, the assumed birth date of the animal and the date of capture (Figure 7).

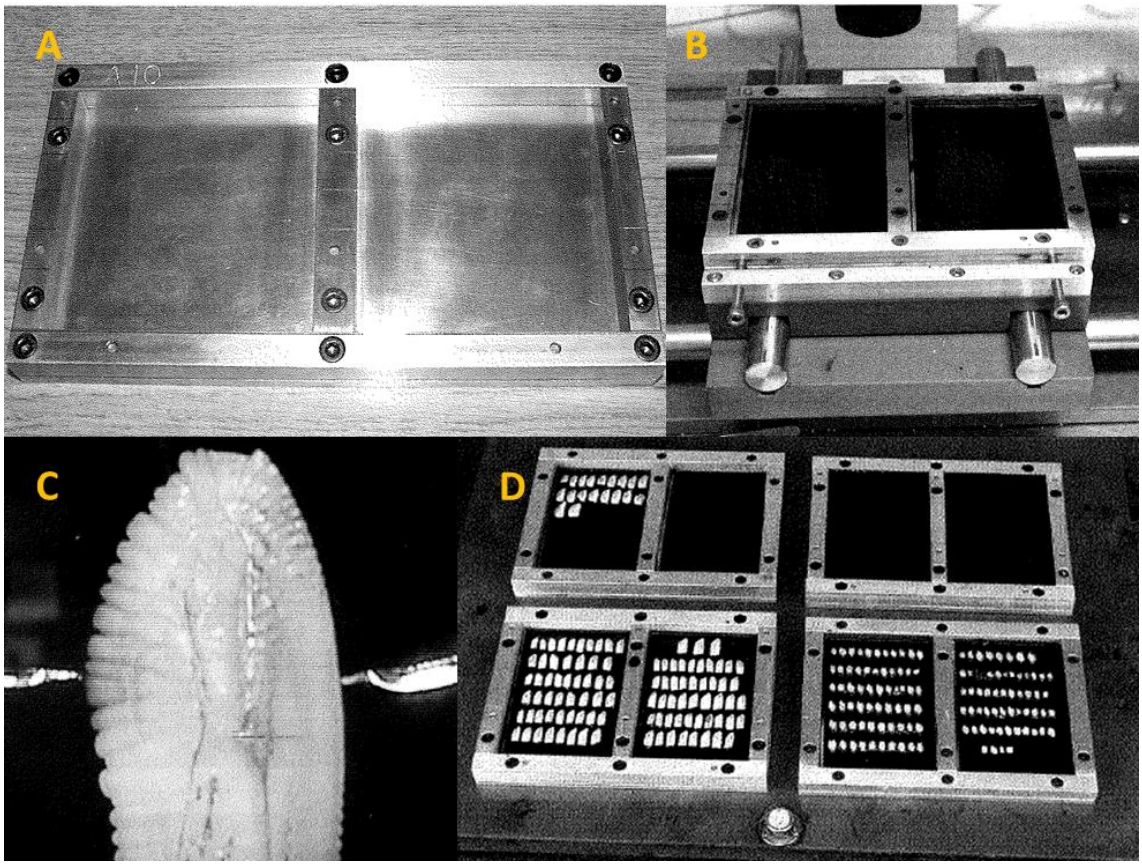

Figure 6. Figure shows otolith embedding equipment including the otolith mould (A), and the mounting jig (B). Image C shows the alignment of the blade guides with the "V" or "waist" of the otolith sulcus (cod), and image D shows the positioned otoliths being left to dry on a level surface.

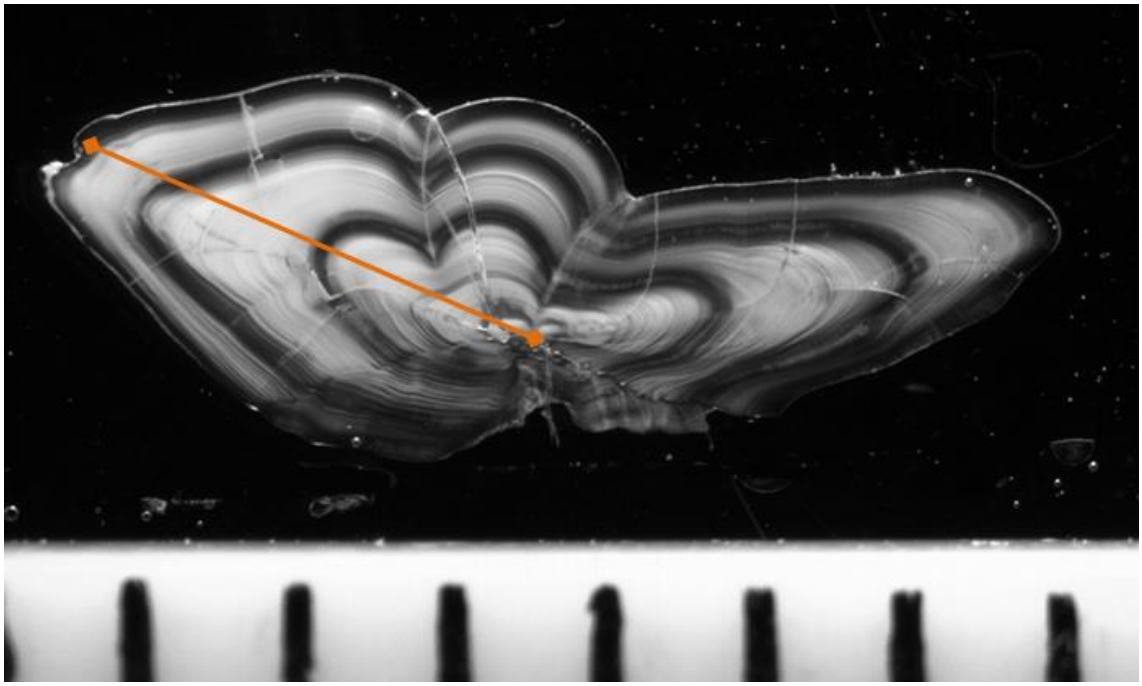

Figure 7. Sectioned otolith (cod) under transmitted light. The line indicated the reading axis used, and the scale bar is in mm. This cod is two years old as there are two opaque rings present.

ANNEX 3: MATURITY ASSESSMENT

Maturity is an important life history parameter to assess as it provides information on trends and seasonal reproductive patterns and is the basis on which we identify the size and age of reproductively active fish. Maturity can be assessed visually, but histological examination of the gonads will facilitate a better understanding of the reproductive cycle and verify the visual assessment.

Visual assessment of maturity stage (and sex)

Although more advanced and species-specific maturity keys have been developed for certain species, and should be used where available, there are a number of generic keys which can be used as a guide to assessing maturity for most fish species. The key developed by Brown-Peterson et al., 2011 is one such key which is widely used; this is outlined in the Table 3 below.

Table 3. Maturity key adapted from Brown-Peterson et al 2011.

Phase	Description(females)	Description (males)
1. Immature	Small ovaries, often clear, blood vessels indistinct	Small testes, often clear and threadlike. Often difficult to distinguish from females at this stage
2. Developing	Enlarging ovaries, blood vessels becoming more distinct	Small testes but easily identified.
3. Spawning capable	Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically	Large and firm testes. Milt may be released under slight pressure
4. Regressing	Flaccid ovaries, blood vessels prominent.	Small and flaccid testes, no milt release with pressure.
5. Regenerating	Small ovaries, blood vessels reduced but present. Ovary wall may be thickened.	Small testes, often clear and threadlike. May be bloodshot or flabby compared with stage 1

Histological assessment of maturity stage

Histological examination of the gonads is used to verify the visual assessment as well as providing a more detailed picture of the reproductive cycle. This assessment is only required for a sub-sample of fish as the process is both costly and time consuming.

Fixation

Gonad samples collected during sampling should be immediately placed in a fixative solution to preserve the structure and composition of the sample and prevent degradation. Typically, a solution of 10% neutral buffered formalin (NBF) is the fixative used for gonad and other soft tissues.

A section of gonad tissue, ~ 1 cm3 is placed in a vial of 10% NBF and left to fix over a minimum period of 24 hours 4°C. This fixative is quite stable and samples can be stored cold in NBF for some time before processing. The ratio of tissue to fixative should always be 1:10+ to ensure adequate fixation.

Processing

Once samples are fixed they can then be processed to remove water and embed the tissue in paraffin wax.

Samples are first trimmed to ensure a flat surface for sectioning and placed in a labelled cassette. The cassettes can be stored in a beaker of NBF while the samples are being prepared. The cassettes are then placed in a processor following the protocol (adapted to be left overnight) outlined in Table 4.

Table 4. Histological processing protocol.

Step	Reagent	Temperature (°C)	Immersion Time (hrs:mins:secs)
1	NBF (10%)	Ambient	0:30:00
2	Ethanol (75%)	Ambient	1:00:00
3	Ethanol (85%)	Ambient	1:30:00
4	Ethanol (95%)	Ambient	1:30:00
5	Ethanol (100%)	Ambient	4:30:00
7	Xylene	Ambient	2:00:00
8	Molten Wax	60	4:30:00
9	Molten Wax (holding step)	60	--:--:--

Embedding

Once samples have been processed, they are then embedded in a wax block ready for sectioning. This is achieved by surrounding the tissue with molten wax and setting in place.

The cassettes containing the tissue samples are removed from the processor and maintained in a container of molten wax. To embed, a mould of appropriate size for the samples is first heated using a hotplate. A few drops of molten wax are added – just enough to thinly coat the base of the mould. The sample is then taken from the cassette (the lid can be stored at this point for re-use) and orientated so that the surface to be sectioned faces down. This must be done gently to avoid compressing the tissue and compromising the cell structure within. When the sample is correctly orientated the mould is transferred briefly to a cold plate (this step helps to fix the sample in place and stop it from floating and turning when the rest of the wax is added to the mould). The cassette is then placed on top of the mould, the mould is filled with wax and transferred to a cold plate to set.

Once the wax is fully set, the blocks are removed from the moulds and excess wax around the cassette is scrapped off with a knife.

Blocks can then be stored in a cool place ahead of sectioning.

Sectioning

To section, blocks are first cooled on a cold plate. Each block is then transferred to a rotary microtome and trimmed at 8-10µm until the tissue is entered. The cutting thickness is then changed to 2-3µm and the desired sections are then removed to a water bath (~40°C) to relax. The section is the picked up with a slide and the slides are transferred to an incubator (60°C) to dry completely before staining.

Staining

Sections are stained to allow for differentiation between tissues. Typically for gonad staging, sections are stained with Mayer's Haematoxylin and Eosin Y following a modification of the procedure of Bancroft and Stevens (1991). The steps are given in Table 5.

Tissue sections are dewaxed and then rehydrated through a series of ethanol dilutions and a final step in flowing tap water. The sections are then over-stained in Haematoxylin. The excess stain is removed by washing in running tap water and the slides are differentiated by dipping in Acid alcohol followed by a tap water rinse. The stain is set using Scott's tap water substitute and rinsed before being counterstained with Eosin. Samples are again washed in running tap water before being dehydrated again in ethanol and cleared in Xylene.

The slides are then cover-slipped in resin and left to dry overnight.

Table 5. Step by step staining procedure, adapted from Bancroft and Stevens (1991).

Step	Reagent	Immersion time
1	Xylene	2 mins
2	Ethanol 100%	2 mins
3	Ethanol 95%	2 mins
4	Ethanol 70%	2 mins
5	Running tap water	30 secs
6	Haematoxylin Z	10 mins
7	Running tap water	5 mins
8	1% Acid alcohol	3-5 quick dips
9	Running tap water	5 mins
10	Scott's tap water substitute	2 mins
11	Running tap water	2 mins
12	Eosin	5 mins
13	Running tap water	2-5 mins
14	Ethanol 70%	2 mins
15	Ethanol 95%	1 min
16	Ethanol 100%	1 min
17	Xylene	Until cover slipping

ANNEX 4: ADDITIONAL RESOURCES

- Brown-Peterson, N., Wyanski, D., Saborido-Rey, F., Macewicz, B. & Lowerre-Barieri, S., 2011. **A standardized terminology for describing reproductive development in fishes**. *Marine and Coastal Fisheries*, 3(1), 52-70. Available from http://www.tandfonline.com/doi/full/10.1080/19425120.2011.555724

- Cochrane, K. & Garcia, S. (eds), 2009. **A fishery manager's guidebook**. Second edition. FAO, Rome; 544 pp. Available from http://www.fao.org/docrep/015/i0053e/i0053e.pdf

- CRFM, 2015. **Draft sub-regional management plan for FAD fisheries in the Eastern Caribbean (stakeholder working document).** *CRFM Technical & Advisory Document 2015/05*. 94p. Available from http://www.crfm.net/images/2015_Draft_Sub-Regional_Management_Plan_for_FAD_Fisheries_in_the_EC_Final_Draft_revised_24_Sept_2015.pdf

- Green, B., Mapstone, B., Carlos, G. & Begg, G. (eds), 2009. **Tropical fish otoliths: Information for assessment, management and ecology.** *Reviews: Methods and Technologies in Fish Biology and Fisheries,* volume 11. Springer, Netherlands; 313 pp. Available from http://www.gbv.de/dms/sub-hamburg/647628791.pdf

- Gezelius, S. & Raakjær, J. (eds), 2008. **Making fisheries management work; Implementation of policies for sustainable fishing.** *Reviews: Methods and Technologies in Fish Biology and Fisheries,* volume 8. Springer, Netherlands; 236 pp. Available from http://www.fcaib.edu.ng/books/Animal%20Production/%5BStig_S._Gezelius%5D_Making_Fisheries_Management_Wor(BookFi.org).pdf

- Stamatopoulos, C., 2002. **Sample-based fishery surveys: A technical handbook**. *FAO Fisheries Technical Paper No. 425.* FAO, Rome; 132 pp. Available from http://www.fao.org/3/a-y2790e.pdf

ANNEX 5: SAMPLING SHEETS

Best Practice

- ⇨ Print all sampling sheets on waterproof paper and always use a pencil when recording data as this will not run if it gets wet.
- ⇨ When writing numbers;
  - Zero = Ø
  - Seven = 7̶
- ⇨ Data should be entered into a database as soon as possible

Completing data sheets

The sampling data sheets consist of three pages; 1) a cover sheet, which should be filled in with details of the trip being sampled, 2) a length frequency sheet for recording the number of individuals from each species at each length class, and 3) a biological sampling sheet for recording details on the samples taken, the weight of the fish and the sex and stage assessed by eye.

Each page should be labelled with the trip/sample ID number and the samplers initials so that they can be matched up if separated for any reason.

The length frequency sheets are designed to be flexible for species of different sizes and time-efficient. There are two versions attached, one for larger fish to be sampled to the nearest whole cm below, and one for smaller species to be sampled to the nearest half cm below. Each species is measured in a separate column which details of the species, the catch weight and the length measurement used. The size range for each species is estimated; when filling in the length frequencies the column gives single or half cm rows from 0 to 9 – the first digit is to be filled in manually. So, for a species sample with an estimated size range between 20 cm and 40 cm, the first 0-9 cm box is skipped (to allow for recording or fish below 20 cm) and a "2" is written beside the next box to indicate that these incremental lengths are from 20 onwards (ie. 20, 21, 22 or 20.5, 21, 21.5). A mark is then placed in the "MEASURED" column for each fish of that length class measured and the marks are summed for each length class in the "TOTAL" column. A mark is also placed in the "OTO" column for any samples which are taken forward for additional biological measurements.

The biological sampling page should be used to record all details taken for individual fish being sampled and details for a given fish should be recorded along the same row and that record should be given a unique ID. This ID should be marked on any samples (otoliths, gonads) taken to allow them to be cross-referenceable with the biological data for that individual. Any additional samples taken (gonad or other tissues) should be noted in the "ADDITIONAL SAMPLES" column.

COVER SHEET

VESSEL ID		DATE	
TRIP ID		TIME OUT	
PORT		TIME IN	
AREA FISHED		DATA COLLECTOR	
GEAR		COMMENTS/NOTES	
GEAR DETAILS			
ESTIMATED TOTAL CATCH			
ESTIMATED CATCH SAMPLED (%)			

PORT		DATE	
TRIP ID		DATA COLLECTOR	
VESSEL ID		COMMENTS/NOTES	
ESTIMATED TOTAL CATCH			
ESTIMATED CATCH SAMPLED (%)			

SPECIES			
CATCH BY SPECIES			
LENGTH MEASURED			SL FL TL
	OTO	MEASURED	TOTAL
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SPECIES			
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PORT	
TRIP ID	
VESSEL ID	
ESTIMATED TOTAL CATCH	
ESTIMATED CATCH SAMPLED (%)	

DATE	
DATA COLLECTOR	
COMMENTS	

SPECIES					
CATCH BY SPECIES					
LENGTH MEASURED			SL	FL	TL
	OTO	MEASURED	TOTAL		
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CATCH BY SPECIES					
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DATE	
DATA COLLECTOR	
TRIP ID	
SPECIES	

OTO ID	LENGTH	WEIGHT	SEX	STAGE	ADDITIONAL SAMPLES

OTO ID	LENGTH	WEIGHT	SEX	STAGE	ADDITIONAL SAMPLES

OTO ID	LENGTH	WEIGHT	SEX	STAGE	ADDITIONAL SAMPLES