Chapter 10

Collection of Data for Fishery Biology Studies and Fish Stock Assessment

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Basic objective of fisheries research is to provide ample information on the status of fish stocks. This information is collected through various sampling procedures and the data are used to provide advice on the sustainable management of fish stocks, upon which the entire fishing industry depends. There are two main sources of data collected and used in fisheries research. These can be divided into fishery independent and fishery dependent data. The first usually involves monitoring the changes in distribution over time in the relative or absolute abundance of fish populations using vessel based surveys in a way that is not subject to the biases inherent in commercial fishery data. The collection and accurate interpretation of both fishery dependent and fishery independent data are of fundamental importance to our understanding of the fished species. Both are needed to gain an understanding of the magnitude of localized changes in fish communities, landings and productivity of the resource. Fishery dependent information involves collection of catch and effort data (CPUE) as well as biological sampling from commercial fisheries. CPUE data are usually collected from logbooks or using direct observation at the site of landings. The underlying assumption is that changes in CPUE accurately reflect changes in the abundance of the fish stocks.

Biological characteristics of the fish catch such as length/age, reproductive and feeding biology are important basic inputs in stock assessment studies. These data can be collected either from commercial fisheries or from resource surveys. It is known that sampling from commercial fishery is subject to many limitations and biases. On the other hand it is the only possibility, for the moment, to be "in touch" with the exploited fish populations. Most of the quantities involved in fish population work can not be obtained or measured throughout the whole population; e.g. it is virtually impossible to measure all the fish caught. Therefore, a part, or a sample, of the population is collected. A good sampling can not be set up until something is known about the variability of the data and how the precision and reliability of the results are affected by the sampling deficiencies and other sources of uncertainty.

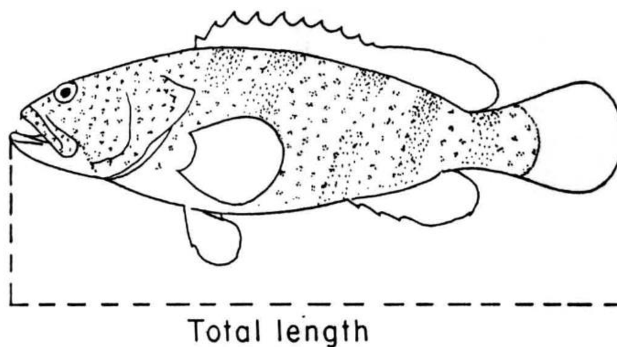
The critical assumption is that a reasonable estimate can be obtained of the true value of the sampled population. The fundamental feature of any sampling system is to collect the data in a random way. Random sampling can be defined as a sampling from some population where each entry has an equal chance of being drawn. In practical terms this means that any fish from the stock under investigation should have the same probability of being sampled. Indeed, this condition is hardly fulfilled. However, the aim to achieve it should always be present in any sampling action. Length measurements of a large representative sample can be taken from the landing centre itself. For biological studies, wherein more tedious effort is required, a small representative sample have to be collected at random and transported to the laboratory in ice boxes.

LABORATORY ANALYSIS

Fish samples previously collected at the landing centre, and stored in the ice box in separate plastic bags, should be analyzed preferably as soon as possible i.e. the same day. This is almost compulsory for the fishes, which spoil easily, to facilitate the collection of data such as sexual maturity stages. The data pertaining to biological characteristics are collected from individual fishes and recorded separately in the data sheets.

Length measurements

Individual Total Length (TL) measurements - from the tip of the snout to the tips of the largest caudal fin rays - are made with the fish placed on its right side, snout to the left, on the measuring board, against the headboard the snout of the fish with its mouth closed is gently pressed. Holding the fish in position with the left hand, its body and tail are straightened along the midline with a single stroke movement using the right hand, and the reading is taken from the measuring ruler. The method of taking length measurement varies from species to species. Usually the maximum length from the tip of the snout to the longest ray of the caudal fin, either upper or lower, as the case maybe, is considered as the total length of the species. However, in certain cases, for example threadfin bream in which the upper lobe is extended into a filament which is likely to be broken, the lower lobe is taken for measurement of the total length.



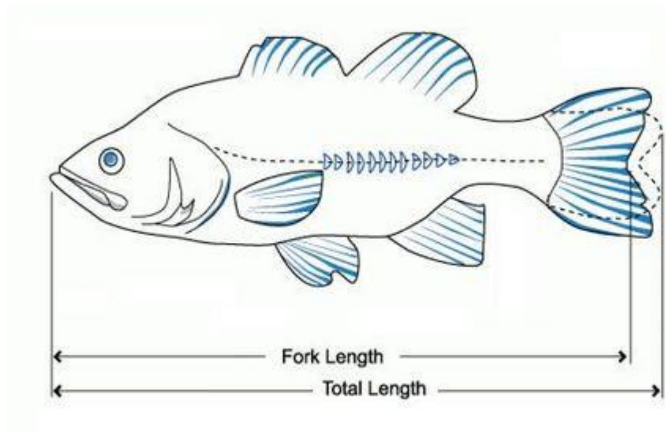


Fig. 1. Length measurements of demersal teleost fishes

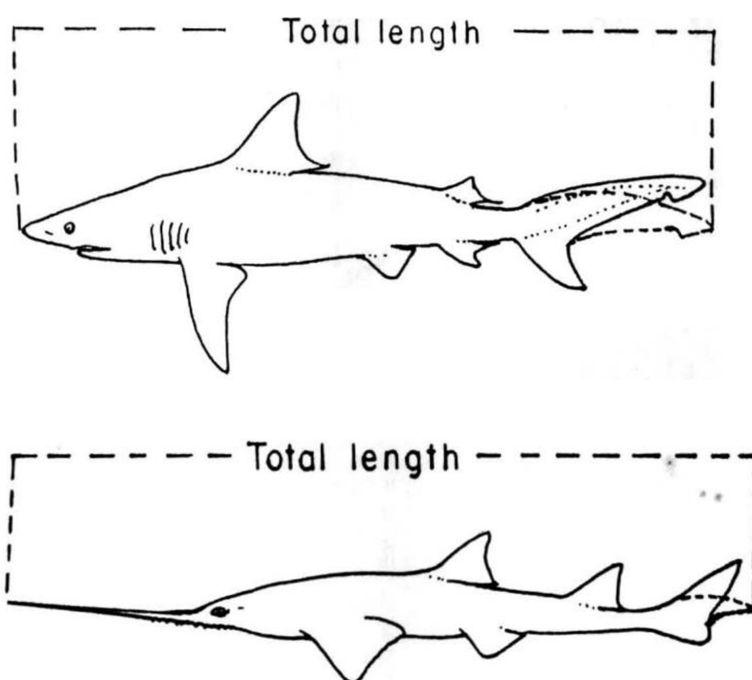


Fig. 2. Length measurements of demersal elasmobrach fishes

In rays and other dorso-ventrally flattened fishes, disc width (Fig. 3) rather than total length is generally used.

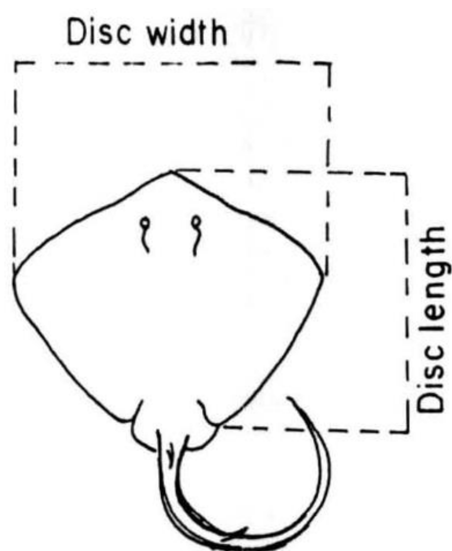


Fig. 3. Length measurements of dorso ventrally flattened fish-Rays

For all length measurements it is essential to have a convenient measuring device. A standard type of measuring board consisting of a flat wooden or plastic platform with a scale centrally fitted on it with a headpiece at the zero end of the scale is useful in field and in laboratory. To measure a fish its mouth is closed, placed in its right side, snout to the left on the measuring board. The snout is pressed down gently at the zero end of the measuring board and the body and tail straightened along the midline before reading is taken from the board.

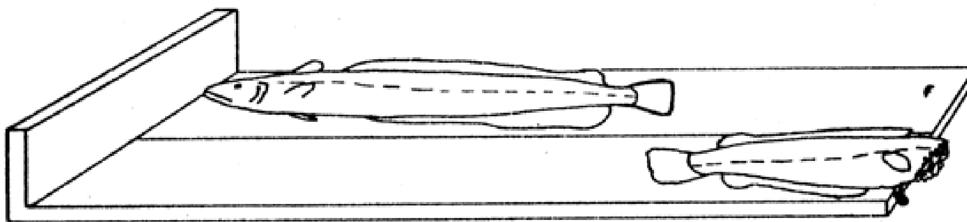


Fig. 4. A graduated measuring board, which is generally used to measure the total length of the fish

Alternatively the length measurements can be taken using high precision scales such as digital Vernier calipers with high sensitivity. Length of large fishes also can be measured using the advanced methods such as laser encoders. With a typical measurement accuracy upto ± 0.05% and equipped with a quadrature output, the Laser-Encoder is a perfectly suited gauge to replace length measurements using other methods. A laser distance meter works by use measuring the time it takes a pulse of laser light to be reflected off a target and returned to the sender. This is known as the "time of flight" principle, and the method is known either as “time of flight” or “pulse” measurement. The distance between the meter and target is given by D=ct/2, where c equals the speed of light and t equals the amount of time for the round trip between meter and target. Given the high speed at which the pulse travels and its focus, this rough calculation is very accurate over distances of feet or miles but loses accuracy over much closer or farther distances. The laser measuring device can be fitted on a

measuring board with a movable reflector piece will help to measure large fish specimens such as tuna, seerfishes and sharks.

Fig. 5. High precision digital Vernier calipers for fish length measurement

After recording lengths from a large set of measurements, a length frequency distribution table may be prepared. The length should be grouped into corresponding length groups and the number falling in each interval is called the frequency of the class interval. The length data should not be combined into large or narrow groupings. A range of about 20 groupings is fairly sufficient for most purposes. The length groupings can usually be 50 mm for species which grow larger than 500 mm, 10 mm for species which grow larger than 200 mm, and 5 mm for species which do not reach 200 mm. The midpoint of the length group is important for the calculation of mean, standard deviation etc.

Fig. 6. Laser measuring device which can be fitted on measuring board for fish length measurement

Weight measurements

For weight measurement of individual fishes, total wet body weight is to be recorded to the nearest 0.1 g on a top-loading balance. Weights of various parts of the fish may be required for biological studies; stomachs for the determination of food eaten, gonads for the estimation of egg numbers and livers for liver-condition assessment.

Fig. 7. Electronic weighing balance for fish weight measurement

The individual weight of each specimen is then recorded in the data sheet against their respective lengths. The length and weight data thus collected will be utilized to fit the length weight relationship, which usually expressed by the equation

$$W = aL^b$$

Where W is the weight of the fish, L is the length and 'a' and 'b' are constants

Sex determination

Sex of the individual fish is determined by gross appearance of gonadal structures. Maturity stages of each gonad are recorded on the basis of morphological appearance (macroscopic observations) such as colour, texture and degree of vascularisation in both the sexes. However, in the case of females, microscopic observations also may be used. Representative gonads were randomly taken from each stage of gonadal development to measure diameter of oocytes. At least 50 oocyte samples from anterior, middle and posterior of the ovary were measured by using a stereo microscope. Intra-ovarian oocyte diameter is measured using an ocular micrometer. Oocytes are grouped in to three to

five OMD intervals. Oocyte diameter from the ovaries of same maturity stages are pooled and plotted to study ova development.

After ascertaining the sexes, the sex ratio is calculated for different months and size groups of fish and is tested for equality for using Chi-square test. Sex ratio is mostly represented by Male: Female (M: F) ratio.

The whole gonad is weighed to the nearest 0.01 g on a digital analytical balance. The weight of the gonad relative to body weight, the gonado-somatic index (GSI), was calculated using the formula:

$$GSI = \frac{\text{Weight of ovary}}{\text{Weight of fish} - \text{Weight of ovary}} \times 100$$

Histological procedure

Part of the gonad identified may be stored for detailed histological analysis. For this, samples of the central portion of the gonads of 0.5 cm thickness are washed, and preserved in 10% neutral buffered formalin (NBF), which is then dehydrated in an increasing ethanol series, n-butilic alcohol, embedded in paraffin and sectioned for 7-10 µm in thickness using a rotary microtome. The sections were stretched in a water bath (40 °C) of distilled water. Three replicate section samples were gathered with the object glass properly labelled and dried for 24 hours at 37 °C followed by one hour at 60 °C over a stove. Sections were stained with a solution of Ehrlich haematoxylin and eosin for a general assessment of the histological components of the gonads.

Sex determination of elasmobranchs

The sex of elasmobranchs can always be determined from external characters because male fish have a pair of mixopterygia (intermittent organs, claspers) which are visible from an early stage of development on the inside edge of the pelvic fins (Fig. 6). The females do not have mixopterygia.

Analysis of Sexual maturity

In most of the demersal teleost fishes, identification of six or seven maturity stages is carried out. They are; Stage 1- Immature, Stage 2- Maturing 1 or spent recovering, Stage 3- Maturing 2, Stage 4- Mature, Stage 5- Ripe/Running and Stage 6- Spent.

Table 1. Description of different maturity stages of demersal teleost fishes

Stage of Maturity	Male	Female	
	Nature and extent of testis in body cavity	**Nature and extent of ovary in body cavity**	**Appearance of ova under microscope**
I Immature	Small, transparent, pale, occupying a very small portion to 1/3 of body cavity	Small, transparent, pale, occupying a very small portion to 1/3 of body cavity, ova not visible to naked eye.	Irregular, small, yolkless/yolk deposit just started, transparent with clearly visible/partially visible nucleus
II Maturing 1/Spent recovering	Whitish, translucent, occupying about 1/2 of body cavity	Pale yellow, granular ova visible to naked eye, occupying about 1/2 of body cavity	Medium sized, assume round shape, opaque, with fair amount of yolk
III Maturing 2	Creamy white, occupy about 3/4 of body cavity	Pale yellowish, blood vessels visible on dorsal side, ova clearly visible, occupying about 3/4 of body cavity	Medium sized, opaque, fully yolked
IV Mature	Creamy white, soft, occupying about full length of body cavity	Pinkish yellow, blood vessels prominent, large ova prominently visible, occupying about full length of body cavity	Large sized, mature, transparent at periphery
V Ripe/Running	Bright creamish, soft and swollen occupying full body cavity	Reddish with fully packed ova visible to naked eye, occupying full length of body cavity	Large sized ova fully packed in the ovary
VI Spent	Flabby, little reddish, occupying about 1/2 of body cavity.	Flaccid, reddish, occupying about 1/2 of body cavity	Medium sized ova present with disintegrating ripe ova

The identification of sexual maturity stages is particularly easy in fresh specimens; however table/hand magnifying lenses have to be used to make the analysis less tiring and time demanding. Initially, when uncertainty arises because of the small size of the specimens and their gonads, then there is only one thing to do to determine the sex: examine the gonads using dissecting microscope.

Steps for the dissection of demersal fishes for biological data collection

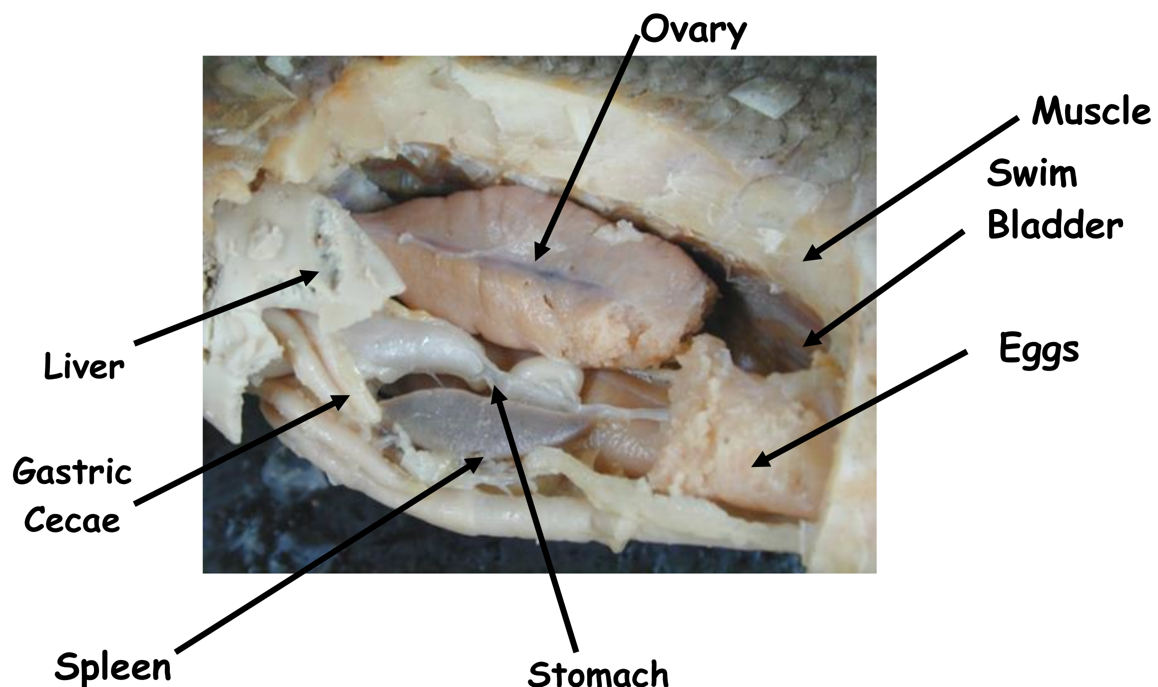


Fig. 8. Close up picture of the body cavity of a demersal teleost fish, showing ripe gonad and other internal organs.

- Remove operculum with scissors
  - Observe gill anatomy
    - Rakers - white, comb-like arches
    - Filaments - Red fingerlike projections
- With a scalpel, remove a section of the lateral line

- Begin the main incision
    - Open the abdomen (below the gill) carefully with a scalpel
    - Cut with a scissors: remove a oval-shaped piece of skin (only skin) running from underneath the gills, to the anus, up to the lateral line, along the lateral line, to the gill, down to where you started the incision. Remove flap of skin (see diagram on next slide)
- Only cut through scales, muscles, and skin
- Take special care not to cut too deep!

Fishes belonging to the maturity stage 4 onwards are considered as mature fish and used for determining the size at first maturity. For this, the fishes belonging to different length groups are to be selected at random for the determination of maturity stages. The length at which 50% of fish are mature is considered as size at first maturity.

The logistic equation used for this is: $P = 100 / (1 + \exp(-r (L-Lm)))$

Where P = percent mature fish in length class L, r = the width of the maturity curve and Lm = length at 50% maturity.

Maturity stages of elasmobranchs

The maturity of males can be easily and best defined from the state of development of the mixopterygia. These of immature fish are small and flaccid and do not reach the posterior edge of the pelvic fin (Fig. 6a). In maturing fish, the mixopterygia are larger; they extend to the posterior edge of the pelvic fins and the internal structure is visible but soft and not ossified (Fig. 6b); in mature fish the mixopterygia extend well beyond the posterior edge of the pelvic fin, the internal structure is visible and is hard and ossified (Fig. 6c).

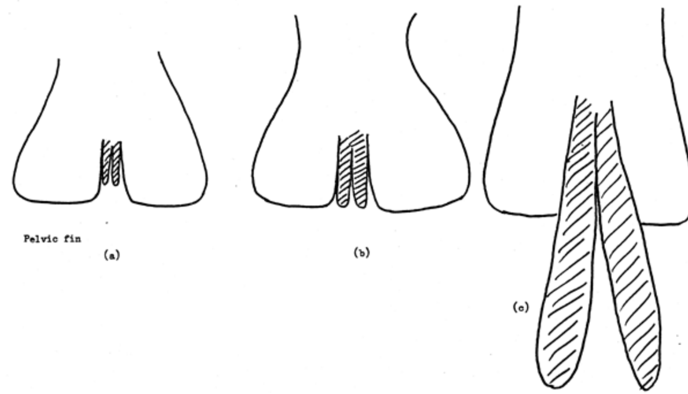


Fig. 9. Maturity stages of male elasmobranchs; mixopterygia are cross hatched. (a) immature, (b) maturing and (c) mature

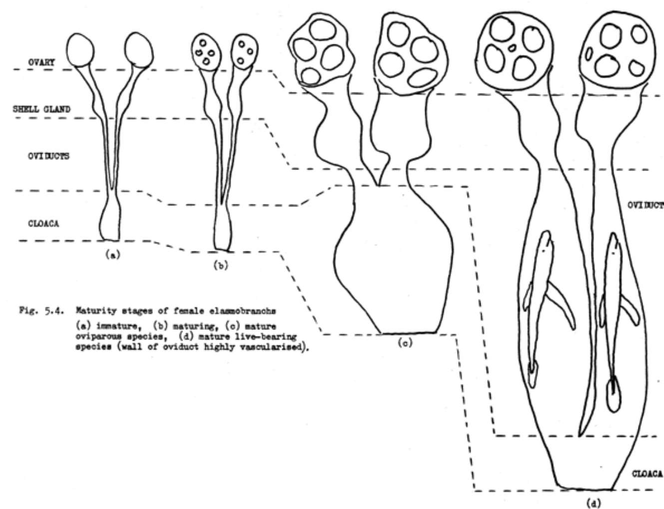


Fig. 10. Maturity stages of female elasmobranchs (a) immature, (b) maturing, (c) mature oviparous species, (d) mature live-bearing species (wall of oviduct highly vascularised).

Maturity of females must be determined by internal examination. The reproductive system of females consists of ovaries (usually two but in some species one only is present), shell glands and oviducts (Fig. 7). In immature fish the ovary is barely discernible and it contains no eggs; the shell gland is also very small and the oviducts are thick-walled and white (Fig. 7a). In maturing fish, white eggs are visible in the ovary but the remainder of the reproductive system is similar to that of immature fish (Fig. 7b). In

mature fish, the ovaries contain yellow eggs, except immediately after ovulation in viviparous species and at the end of the spawning season in oviparous species; the shell gland is enlarged and the oviducts distended and, in viviparous species, thin-walled, flaccid and often highly vascularized (Fig. 7d). In viviparous species maturity is also associated with changes in the size of the cloaca (Fig. 7c).

Sampling the Otolith fish ageing

Age of fishes is estimated using a small bone found in the head of fish called the otolith. Counting the rings on these otoliths gives an estimate of the age of the fish (similar to the rings of a tree). Otoliths, commonly known as "earstones," are hard, calcium carbonate structures located directly behind the brain of bony fishes. Otoliths are popular because compared to other structure they generally provide the most accurate ages, mainly due to their continued growth throughout the life of the fish. Otoliths provide an abundance of information ranging from temperature history, detection of anadromy, determination of migration pathways, stock identification, use as a natural tag, and most importantly age validation.

Different species have otoliths of different shapes and sizes; and cartilaginous fishes, such as sharks, skates, and rays, have none. This figure shows the growth rings of a sagittal otolith section viewed under reflected light. The dark translucent zone represents a period of fast growth. The white opaque zone represents a period of slower growth. Biologists estimate fish age by counting these opaque zones, called annuli, just as one would count rings on a tree to determine its age.There are three types of otoliths, all of which aid fish in balance and hearing:

1. Sagitta: The largest of the 3 pairs of otoliths, sagitta is involved in the detection of sound and the process of hearing, or converting sound waves into electrical signals
2. Asteriscus: This type of otolith is involved in the detection of sound and the process of hearing.
3. Lapillus: This type of otolith is involved in the detection of gravitational force and sound (Popper and Lu 2000)

Otoliths, as well as other boney structures form yearly rings (similar to that of a tree) known as annuli. Each annulus is composed of opaque and translucent zones, which correspond to periods of fast and slow growth (figure 1). The most common method of age validation is marginal increment analysis. The marginal increment is the measurement from the last annulus to the margin (or edge of the otolith). Each graph above represents the average monthly marginal increment values for one year for ages one through four. The marginal increment cycles down only once during each year, which means that one annulus was deposited once per year.

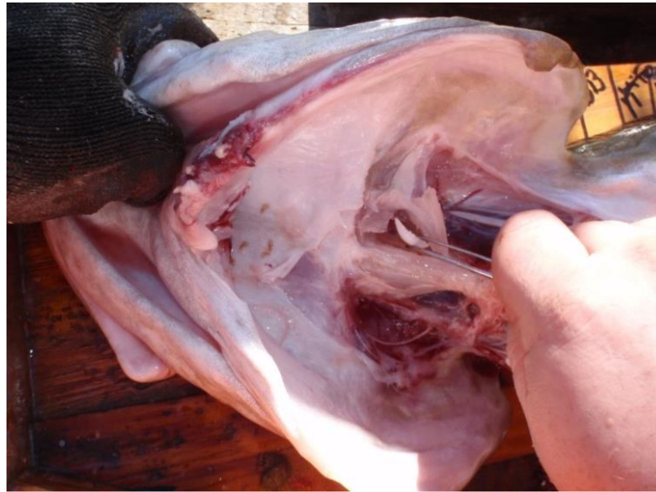


Fig. 11. Removal of otolith from teleost fishes

Otolith removal procedure

1. Cut the operculum to fold forward and open it wide towards the anterior end of the fish.
2. Cut away the gill arches at their insertion.
3. Use a chisel to scrape away tissue from the otolith capsule, the capsule will feel like a large knob or protrusion.
4. Open the capsule with a chisel, the large sagittal otoliths can be easily removed with forceps.

5. Rub off any attached membranes from the otolith, rinse with fresh water and pat dry.
6. Place otolith in the paper envelope.

Each label should be filled with the appropriate fishery information. Use a soft lead pencil on the label. Alcohol and water will dissolve ink and sample information will be lost. Wash otoliths off with water, using the supplied spray bottle and dry each otoliths before placing them in sample centrifuge tube.

The otoliths will not degrade easily. Avoid putting them in fixatives like formalin which can become acidic over time and lead to dissolution of the otoliths. In the fisheries labs they are usually stored in small paper packets which can then be labeled and stored very easily. If there is any chance you might want to do any microchemistry on the otoliths later on, you have to be more careful to avoid contamination from metal forceps etc.

Collection of data for fecundity estimation

Knowledge of the fecundity of a species is an important factor in fish stock management. It is used to calculate the reproductive potential of a stock and the survival from egg to describe a fish which is spawning for the first time. For fecundity estimation, female gonads belonging to the stage 5 are used. After ascertaining the sex and maturity stages, portions are cut from the mid-region of the ovary, weigh to the nearest 0.01 g, and place in Gilson's fluid for fecundity estimates. Gilson's fluid is prepared by mixing 100 ml 60% alcohol, 15 ml 80% nitric acid, 18 ml glacial acetic acid, 20 g mercuric chloride in 800 ml of water. The material for fecundity estimates can be stored in Gilson's fluid for 3 months.

The plastic bottles containing the material are vigorously shaken from time to time to aid in the release of oocytes from the ovarian walls. Before counting, the contents of each bottle are to be poured into a petridish and those oocytes not liberated from the ovarian tissue removed by teasing. The oocytes were repeatedly washed in tap water. The clean

and separated oocytes were transferred to another 1 litre beaker containing a known volume of 1 molar sugar solution. A plastic ruler can be used to stir vigorously the egg suspension to ensure an even distribution of oocytes in the suspension column. After 10 strokes of the ruler a sub-sample was taken by a pipette. One aliquot will usually give sufficient numbers of large and small oocytes to yield satisfactory counts and diameter distributions. The oocytes are pipetted into a counting chamber, and their diameter should be measured along a horizontal axis using a calibrated eye-piece ocular micrometer under a standard dissecting microscope at a magnification of 40X. The accuracy of the sub-sampling method was tested by taking 10 replicates and calculating the coefficient of variation which was found to be 5.8%. The fecundity (*F*) for each female fish was calculated as follows:

$$F = \frac{V}{V_1} n \times \frac{W}{W_1}$$

where, n = number of oocytes in the sub-sample; V = volume of the egg suspension; V1 = volume of sub-sample; W = weight of whole ovary; W1 = weight of portions of ovary fixed.

The relationships between total length of fish (TL) and fecundity (F), total weight of fish (TW) and fecundity (F) and ovary weight (OW) and fecundity (F) were estimated by linear regression analysis based on the equation,

$$\text{Log } F = \text{Log } a + b \text{ Log } X$$

Table 2. Record sheet for collecting biological data on demersal finfishes

Species:			Landing Centre:				Date:		
Sl. No.	**Length (mm)**	**Weight (g)**	**Sex (M/F)**	**Maturity (1-6)**	**Gonad wt (g)**	**Stomach condition**	**Stomach wt (g)**	**Stomach Contents**	**Remarks**

Table 3. Sample data sheet for tabulation of biological data after analysis.

CENTRAL MARINE FISHERIES RESEARCH INSITUTE

Project Code: **P-3a**

Period: January -December2010 **Centre: Cochin Fisheries Harbour** **Resource: *S. undosquamis***

Month	Length Range (mm)	Modes (mm)	Mean Size (mm)	Sample size(nos.)	Sample weight(kg)	Sex ratio M:F	Immature%	Mature(%)	Gravid/ Spent (%)	Sample size(nos.)
Jan	201-300	235,245,285,	240	36	4.074	1:1.6	0	0	0	0
Feb	191-290	215,235,	230	39	4	1:2.1	0	0	0	0
Mar	161-300	215,255	227	76	6.235	1:2.5	43	11	46	28
April	181-330	195,215,225	232	47	5.355	1:12.6	80	10	10	39
May	131-340	215,225	224	69	6.415	1:3.3	94	0	6	53
June	171-320	245,255	234	61	5.241	1:0.8	75	18	7	28
August	201-290		248	79	6.577	1:1.6	100	0	0	24
Sept.	211-300	265	251	36	5.087	1:1	11	50	39	18
Oct.	171-330	235,255,275	236	61	6.232	1:1.2	10	72	18	33
Nov.	141-300	165,235,245	221	82	7.029	1:1.7	14	45	24	29
Dec.	171-310	235,275	242	82	9.47	1:1	6	77	18	17
Annual	131-340			668	65.45	1:2.7				269

Table 4. Tabulation form for collecting data on fecundity of fishes

Sl. No.	Length (cm)	Total weight (g)	Ovary wt (g)	Fecundity (Nos.)

Collection of data on stomach contents

The simplest measurement for gut contents is occurrence frequency, as it demands only the stomach food observation. The other best known methods are the Relative Importance Index, and the Feeding Index. For all of them, the principle involved is that food items should be counted or, at least, weighed or measured by their volume. When the fish is cut open for the examination of the gonad maturity condition, the stomach contents also examined. The stomach condition is determined by the degree of

distension of the stomach and classified as Gorged, Full, ¾ full, ½ full, ¼ full, trace and empty. The above classification gives the intensity of feeding and the empty, trace and ¼ full stomachs are considered as poorly fed and others as actively fed. The gut is then dissected and the contents are removed into a Petri dish for qualitative and quantitative analysis. In many cases, the food matter in the gut, especially the crustacean matter will be found to be in an advanced state of digestion and in such cases only the generic level identification of the food components will be possible.

The total weight of the stomach as well as the individual food components are taken to the nearest mg in an electronic digital balance. The quantitative analysis by volumetric method, the gut contents are sorted out and the total volume of each food item is measured using a measuring cylinder graduated to 0.1 ml.

Other morphometric measurements of demersal finfishes

Historically, the morphology of fishes has been the primary source of information for taxonomic and evolutionary studies. There are numerous characters available for morphological study. These characters are most commonly divided in to two categories: morphometric and meristic.

Morphometric characters refer to measurable structures such as fin length, head length, eye diameter, or ratios between such measurements. **Meristic** characters include almost any countable structure, including fin rays, scales, gill rakers, and so on.