

# **Collection of biological data on demersal resources- practical**

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## **1. INTRODUCTION**

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.

## **2. 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 .

## 2.1 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.

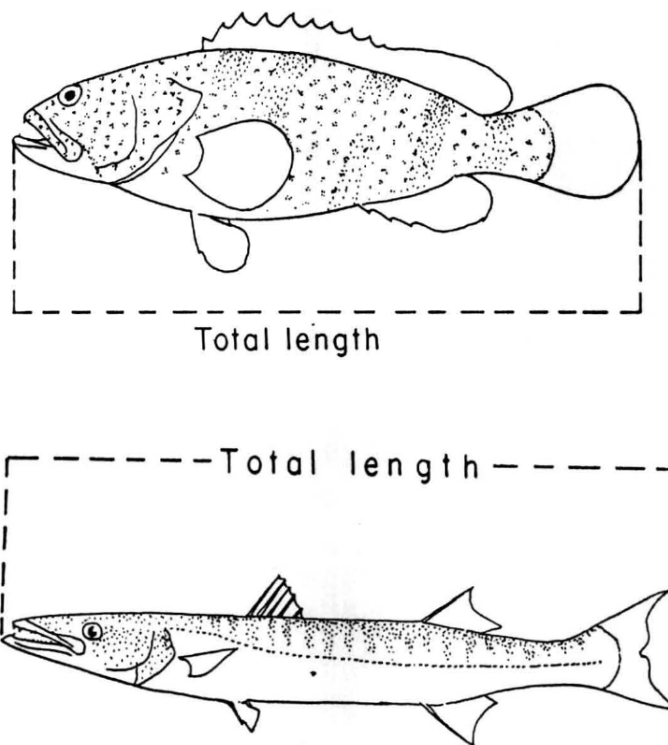


Fig. 1. Length measurements of demersal teleost fishes (Grouper- *Epinephelus tauvina* and Barracuda *Sphyraena obtusata*)

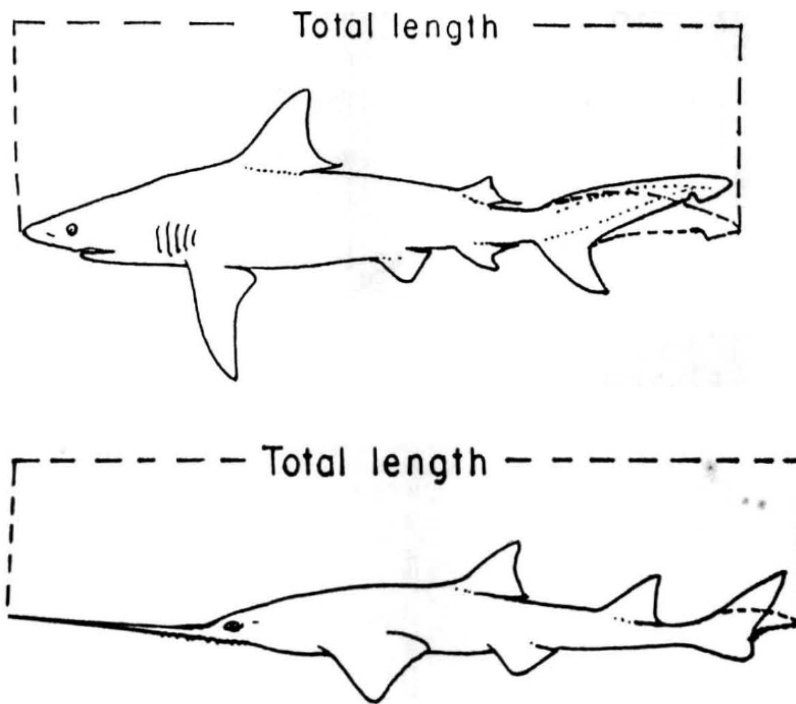


Fig. 2. Length measurements of demersal elasmobranch fishes (Shark- *Carcharinus limbatus* and Skate- *Pristis microdon*)

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

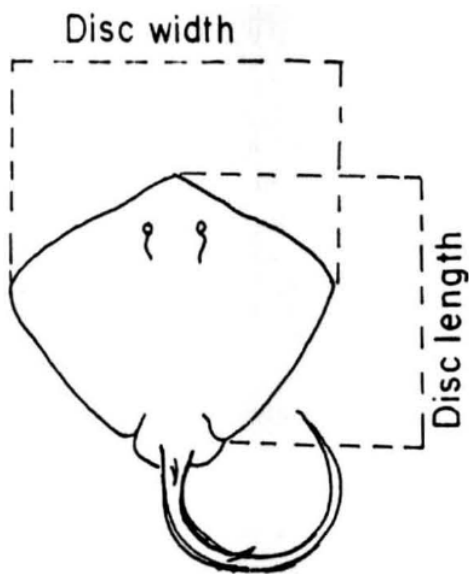


Fig. 3. Length measurements of dorso ventrally flattened fish (Rays – *Dasyatis* sp.)

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.

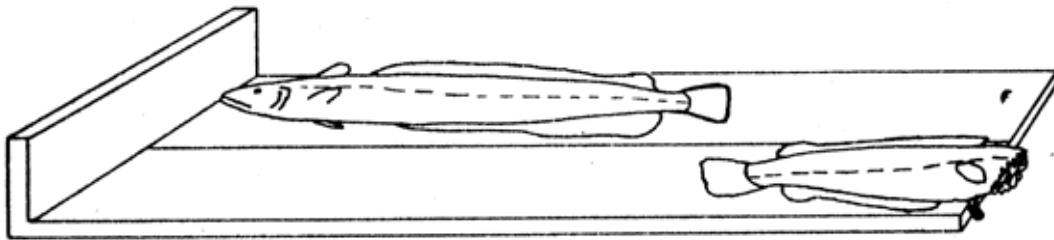


Fig. 4. A graduated measuring board, which is generally used to measure the total length of the fish

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 mid point of the length group is important for the calculation of mean, standard deviation etc.

## **2.2 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.

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

### **2.3 Sex determination**

Sex of the individual fish is determined by gross appearance of gonadal structures. Maturity stages of each gonad is 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 :

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

#### **2.3.1 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-butyl 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.

### **2.3.2 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.

### **2.4 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. 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**

- ▶ 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!

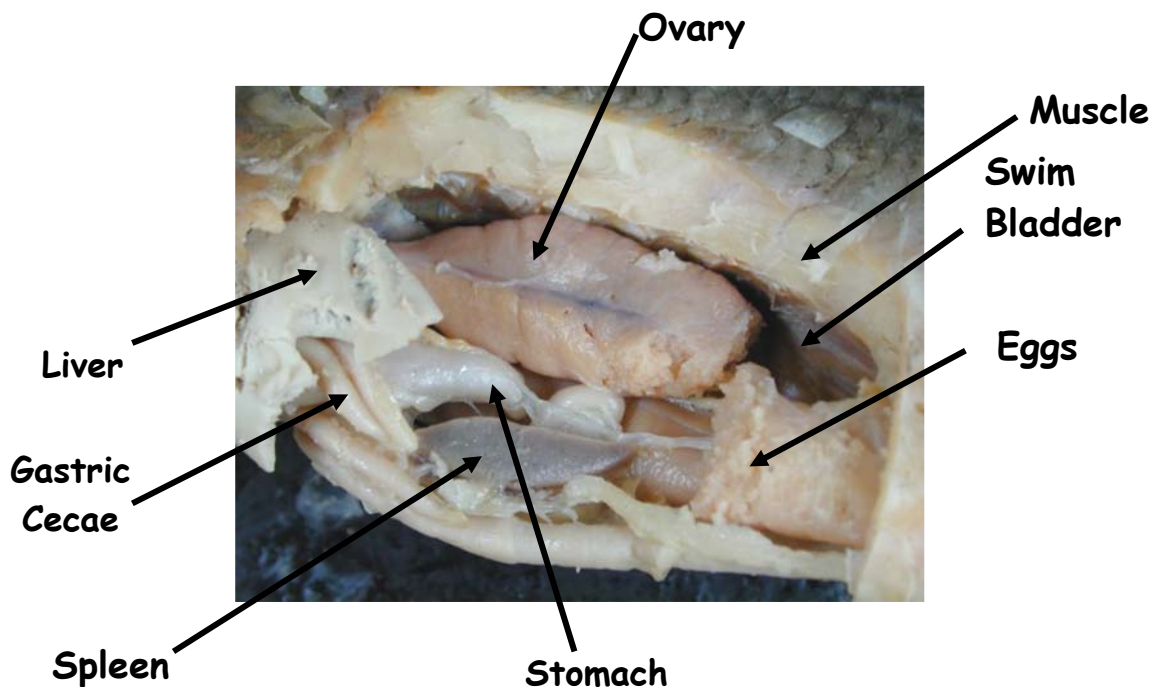


Fig. 5. Close up picture of the body cavity of a demersal teleost fish, showing ripe gonad and other internal organs.

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:  $P = 100 / (1 + \exp(-r(L-L_m)))$

Where P = percent mature fish in length class L, r = the width of the maturity curve and L<sub>m</sub> = length at 50% maturity.

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

#### 2.4.1 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).

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 oviduots (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).

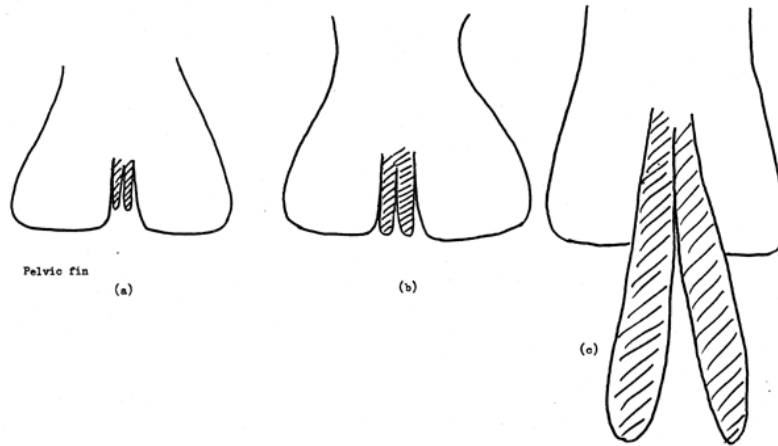


Fig. 6. Maturity stages of male elasmobranchs; mixopterygia are cross hatched. (a) immature, (b) maturing and (c) mature

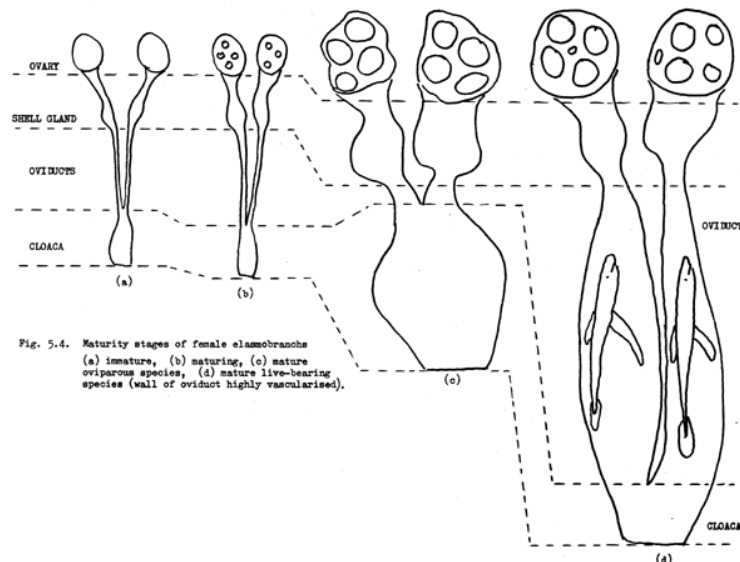


Fig. 7. Maturity stages of female elasmobranchs (a) immature, (b) maturing, (c) mature oviparous species, (d) mature live-bearing species (wall of oviduct highly vascularised).

## 2.5 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 an 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:

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



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.)

## 2.6 Collection of data on stomach contents

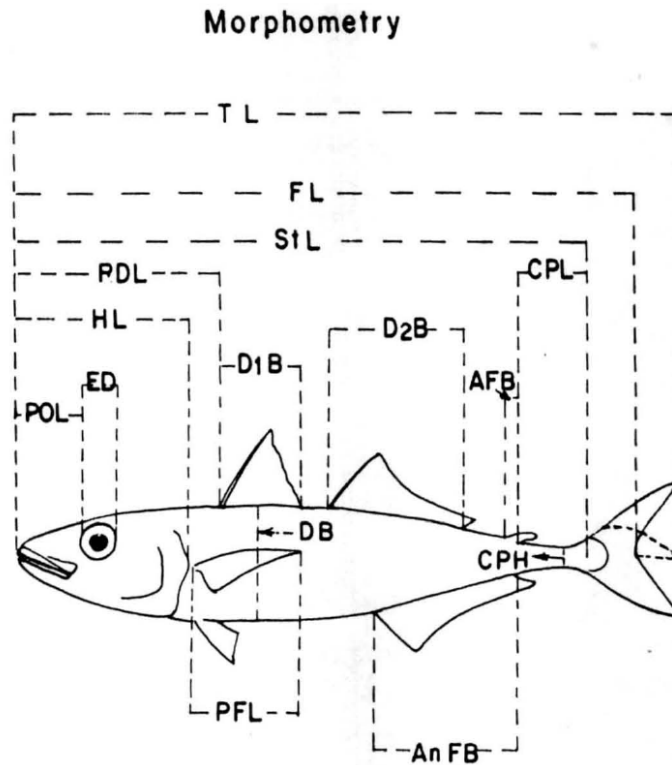
The simplest measurement for gut contents is occurrence frequency, as it demands only the stomach food observation. The other best known methods are the Relative Importance Index, and the Feeding Index. For all of them, the principle involved is that food items should be counted or, at least, weighed 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 Georged, Full,  $\frac{3}{4}$  full,  $\frac{1}{2}$  full,  $\frac{1}{4}$  full, trace and empty. The above classification gives the intensity of feeding and the empty, trace and  $\frac{1}{4}$  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.

## 2.7 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 into two categories: morphometric and meristic.

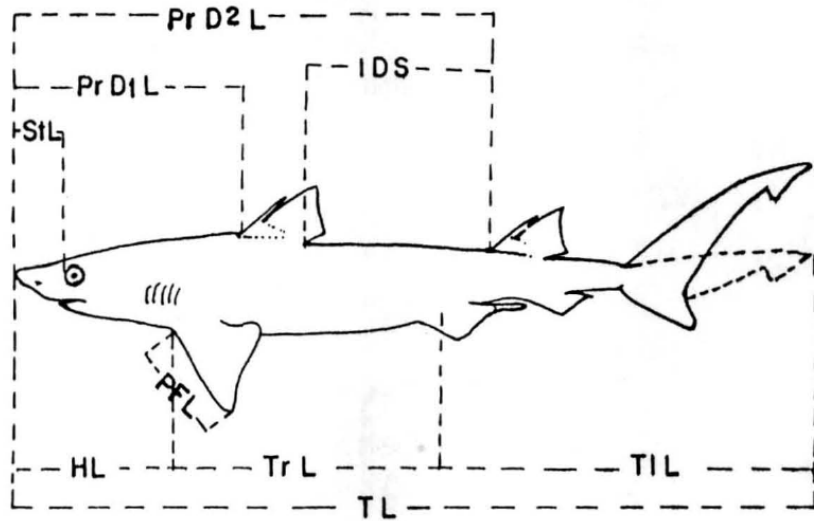
**Morphometric** characters refer to measurable structures such as fin length, head length, eye diameter, or ratios between such measurements. **Meristic** characters include almost any countable structure, including fin rays, scales, gill rakers, and so on.



TL	Total Length
FL	Fork Length
StL	Standard Length
PDL	Pre Dorsal Length
HL	Head Length
POL	Pre Orbital Length
ED	Eye Diameter
D1B	First Dorsal Base
D2B	Second Dorsal Base
CPL	Caudal Peduncle Length
AI"U	Adi pose Fin Base
DB	Depth of Body

CPH	Caudal Peduncle Height
PFL	Pectoral Fin Length
AnFB	Anal Fin Base

Fig. 8. The important morphometric measurements which are used for identification of demersal teleost fishes (eg., croakers)



TL	Total Length
HL	Head Length
TrL	Trunk Length
TIL	Tail Length
StL	Snout Length
Pr. D1 L	Pre Dorsal (1) length
Pr.D2L	Pre Dorsal (2) length
IDS	Inter Dorsal Length
PFL	Pelvic Fin Length

Fig. 9. The important morphometric measurements which are used for identification of demersal elasmobranch fishes (eg., sharks)

