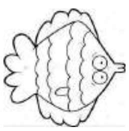
CHAPTER 23



Standardised Protocol for Taxonomic Measurements for Pleuronectiform Fishes

Introduction

Study of fish morphometrics has been the primary source of information for taxonomic and evolutionary studies. Despite the value and availability of genetic, physiological, behavioural, and ecological data for such studies, systematic ichthyologists continue to depend heavily on morphology for taxonomic characters. Morphometric data is important in that it can be used as taxonomic characters to examine evolutionary relationships among species; they have the advantage that size effects can be removed before the data are recoded so that inferred evolutionary relationships are based on body-form rather than body-size differences. Identification of stocks of fish has been the mainstay of morphologists. Large data sets have been collected for a diverse array of commercially important fish (Winans,1985). For over 30 years, most morphometric investigations have based the selection of characters on the set of measurements described by Hubbs and Lagler (1947). Most species of fishes have characteristic shapes, sizes, pigmentation patterns, fin disposition and other external features that aid in recognition, identification, and classification that can be examined by dissection or other means of internal examination. Structural measurements sometimes are used directly as characters if they are sufficiently discrete among taxa or if a tree-building procedure is used that allows the use of continuous characters (Farris 1970; Farris et al. 1970). Standard references for taxonomic study of bony fishes are Hubbs and Lagler (1958), Miller and Lea (1972), Lagler et al., 1977, Bond (1979), Moyle and Cech (1981), and Trautman (1981). The general parameters taken into account are those on the left side for bony fishes unless otherwise mentioned or right side when that side is damaged. In the case of elasmobranchs, a glance through any well-illustrated guide to chondrichthyans (e.g. Compagno 1984; Last and Stevens 1994; Compagno et al., 2005) reveals a huge diversity of body morphology. In odd shaped teleost fishes like box fishes also the basic measurement pattern was based on Hubbs and Lagler (1958) with slight modifications. Compared to the other teleosts, measurements are necessary on both sides for the flatfishes due to the flattened nature of the body like in the ray fishes.

Morphological characters have been commonly used in fishery biology studies to measure discreteness and relationships among various taxonomic categories (Jerry and Cairns, 1998). Morphometric analysis can thus be a first step in investigating the stock structure of species with large population sizes. Study of the morphometric characters are important to understand the interspecific variations among species. Interspecific shape comparisons are best done after an analysis of within species variation has been completed. Intra-species variation has two basic

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components (Barlow 1961) and has been noted for many species (Hubbs, 1922; Taning, 1952; Weisel, 1955; Lindsey, 1958, 1962; Fowler, 1970) and should be taken into account in studies involving meristic characters. Meristic features may also be size-dependent within or among species (Strauss, 1985).

Identification of new species is very important in the present context of the warming oceans and migration and shifting of species to warmer waters. Flatfishes are characterised by their deep bodied unusual flattened shape, larvae with bilaterally symmetrical eyes and presence of both eyes on the same side of the head in juvenile and post-metamorphic individuals, their remarkable ability to match the colour and pattern of their background and to bury deep in the soil with only the eyes protruding out. 678 extant species of flatfishes are recognized worldwide in approximately 134 genera and 14 families. Earlier studies on cynoglossids by Norman (1934), Menon (1977) helped developed a morphometric pattern for data collection; Amoaka (1969) developed a morphometric table for sinistral flounders of Japanese waters which was later modified in the work of Rekha and Gopalakrishnan (2011).

9075 t of flatfishes was landed in Kerala during 2016; landings have shown an increase over the years from 2012; however contribution of *Cynoglossus macrostomus* to the fishery showed a sharp decline since 2012 in Kerala. *Cynoglossus macrostomus* which once formed 98% of the Malabar flatfish fishery has decreased to 78 % of the landings. *Psettodes erumei* the Indian halibut has vanished from the commercial fishery. Studies by Rekha and Gopalakrishnan (2012; 2016) have revealed the presence of 63 species of flatfishes belonging to 8 families and 26 genera in Indian waters. The changing climatic and fishery patterns as well as the natural disasters have been seen to introduce newer fish species into the commercial fishery. For the correct identification of the newer species a standard protocol is very much essential in view of the unusual shape of the fish; hence this paper is attempted.

Procedure

This involves collection of fish from the harbour or lakes and presentation for further analysis. The procedure for handling delicate flounders and soles and strong halibuts are the same. Fish handling and fish preparation for data collection involves a few preliminary steps unlike the other teleosts and elasmobranchs. Care is to be taken to minimize the stress to the animals especially in the case of soles as they exude a lot of slime when obtained live. Flatfishes when collected by trawl loose fins and scales; hence care is to be taken to see that most of the fishes which were collected are in good condition. The fishes are to be packed in ice before being brought to the lab. While packing the fish in ice, they should be placed in horizontal position to prevent the body shape from changing. OHP sheets to be placed horizontally on ice and the flatfishes to be placed on them before the crushed ice is placed on them. Live fishes generally wriggle a lot which causes their body shape to twist leading to *rigor mortis* later. Once the fishes were brought to the lab, they should be thoroughly cleaned to remove dirt and detritus as well as the mucous which laminates the fishes eg. soles when they are stressed. The fishes should be placed on a flat surface preferably on a transparent OHP sheet/plastic sheet with their blind side down. The fins should be spread out using a needle or scalpel so as to preserve them in their natural condition and to facilitate easy counts. They should be injected with 1% formalin in the abdominal region and caudal region; dilute formalin should also be poured onto the body to stiffen the fins in spread out position. Once ready, they are to be stored in wide open mouth bottles, tagged with date of collection, gear and locality and used for further studies. Fishes should be photographed both in fresh condition as well in this preserved stage. Colour in fresh as well as prominent external features/markings is also to be noted immediately.

The side of the body which houses the eye is called the eyed side while the other side is called the blind side. The blind side is also the ventral side of the fish. Measurements are to be made on the eyed side of the psettodid, right side of each soleid specimen and on the left side of the bothid and cynoglossid specimen. In addition, some morphometric measurements like pectoral fin and pelvic fin length, base width, pre-pelvic, pre-pectoral length are to be taken on blind side also. Meristic counts are to be taken on both sides. It is suggested that the measurements presented herewith be taken as the minimal set of measurements for pleuronectiform fishes. Descriptive terms are also provided for description of species. New measurements may be added for morphometrics as well as for descriptions on a case to case basis. Basic flatfish taxonomy follows Amaoka (1969) with the following additions and modifications

Meristic (counts)

1) **Fin count**: All rays whether branched or unbranched were counted as single rays. (D, A, P_1, P_2, V_1, V_2, C where D stands for dorsal fin, A for anal fin, P_1, P_2, stands for the pectoral fin on ocular and blind side, V_1, V_2 for pelvic fin on the ocular and blind side respectively and C for Caudal fin.
2) **Gill raker**: Count was taken for first gill raker on ocular side.
3) **Lateral line count**: The scales of the middle lateral line represented by pores were counted from the first scale above the angle of the gill opening to the scale at the end of the hypural plate on the caudal peduncle. In case of cynoglossids, the scales between the upper and middle lateral lines were also counted in a diagonal line following the natural scale row.
4) **Head scale count**: An oblique row of scales on the head counted posteriorly from the posterior border of the lower eye.

Morphometric measurements (Figs. 1,2)

1) **Total length (TL)**: From tip of snout to the posterior margin of caudal fin.
2) **Standard length (SL)**: From tip of snout to posterior tip of caudal peduncle.
3) **Head length (HL)**: From tip of snout to posterior angle of opercular margin.
4) **Head width (HW)**: Greatest width across head at posterior portion of operculum.
5) **Head depth (HD)**: Distance from anterior origin of operculum to the ventral side of head.
6) **Snout length (SNL)**: Distance between tip of snout and middle outer margin of orbit (taken for both the upper (SNL_1) and lower eye (SNL_2)).
7) **Eye diameter (ED)** (upper and lower): Greatest distance across eye measured parallel to body length (does not include fleshy area) – ED_1 for upper eye and ED_2 for lower eye.
8) **Interorbital distance (ID)**: Narrowest width between two orbits measured vertical to body length.
9) **Chin depth (CD)**: Vertical distance between the end of the maxillary and the most ventral aspects of the head.
10) **Pre orbital (PrOU, PrOL)**: Distance from the tip of snout to the middle point of the orbit; taken for both upper and lower eye respectively.
11) **Post orbital (PBU, PBL)**: Distance from posterior point of orbit to the outer angle of opercular margin
12) **Upper jaw length (UJL)**: Distance from tip of upper jaw to outer free end of maxillary.
13) **Lower jaw length (LJL)**: Distance from inner angle of mouth of outer tip of lower jaw.
14) **Upper head lobe width (UHL)**: Distance from dorsal margin of body to dorsal/upper origin of operculum.
15) **Lower head lobe width (LHL)**: Distance from dorsal origin of operculum to most ventral part of operculum.

16) **Body depth (BD_1)**: The vertical distance across body just in front of anal fin.
17) **Body depth (BD_2)**: Distance across the widest part of the body exclusive of fins measured on ocular side.
18) **Dorsal fin length (DFL)**: The distance from base of the n^{th} dorsal fin to its tip. The n^{th} dorsal fin ray will be the longest dorsal fin ray taken near the middle of the body or near the maximum width of the body. In cases where the first few rays of the dorsal fin are longer, their lengths are taken separately.
19) **Anal fin length (AFL)**: The distance from base of the n^{th} anal fin to its tip. The n^{th} anal fin ray will be the longest anal fin ray taken near the middle of the body or near the maximum width of the body.
20) **Pectoral fin length (P_1FLO, P_2FLB)**: The length of the longest pectoral fin ray; measurements are taken for ocular and blind side separately as size of the fins are found to be different.
21) **Pelvic fin length (V_1FLO, V_2FLB)**: The length of the longest pelvic fin ray; measurements are taken for ocular and blind side separately as size of the fins are found to be different.
22) **Caudal fin length (CFL):** Distance from the hind end of the vertebral column to the maximum length of the caudal fin
23) **Caudal peduncle length (CDL)**: Horizontal distance between last ray of dorsal fin and origin of caudal fin.
24) **Dorsal fin base (DBL)**: Horizontal distance from base of first dorsal fin ray to the last dorsal fin ray. Measurements are taken on blind side when origin of dorsal fin is on blind side.
25) **Anal fin base (ABL)**: Horizontal distance from base of first anal fin ray to the last anal fin ray.
26) **Pectoral fin base (P_1BLO, P_2BLB)**: Vertical distance across the pectoral fin base; measurements are taken for ocular side and blind side.
27) **Pelvic fin base (V_1BLO, V_2BLB)**: Horizontal distance across the pectoral fin base; measurements are taken for ocular side and blind side.
28) **Caudal peduncle depth (CPD)**: Vertical distance from base of last dorsal fin to the base of last anal fin.
29) **Trunk length (TKL)**: Longitudinal distance from posterior angle of operculum to caudal fin base.
30) **Pre dorsal length (PDL)**: Tip of fleshy snout to base of first dorsal ray (measured on ocular/blind side based on position of origin of dorsal fin).
31) **Pre anal length (PAL)**: Tip of fleshy snout to origin of anal fin.
32) **Pre pectoral length (P_1LO, P_2LB)** : Distance from tip of snout to origin of pectoral fin (both ocular and blind)
33) **Pre pelvic length (V_1LO, V_2LB)**: Distance from tip of snout to origin of pelvic fin (both ocular and blind).

Qualitative characters

1) **Eye**: Relative position of upper (migrating) eye and lower (fixed eye) as well as their position on head.
2) **Jaw position**: Relative position of upper jaw with respect to lower eye. The point of the ending of the upper jaw in front of, behind or just below lower eye is also noted. This denotes the length of the upper and lower jaw.
3) **Dentition on upper and lower jaw on ocular and blind side**: Nature and pattern of teeth on both the jaws on both ocular and blind side are noted.
4) **Fin pigmentation**: Presence/absence of characteristic markings on fins or patterns if any.

5) **Body pigmentation**: Presence/absence of pigmentation on body.
6) **Peritoneum pigmentation:** Relative intensity and coverage of pigmentation on the peritoneum; pigmentation varies with different species.
7) **Opercular pigmentation**: Pattern of pigmentation varies on the surface of the operculum.
8) **Membrane ostia:** Presence /absence of membrane ostia (small pores) in the basal part of the membranes of the dorsal and anal fins.
9) **Ocular/ rostral spines:** Presence/absence of spines near/ around eye and snout.
10) **Dorsal fin origin:** Relative position of the dorsal fin on the body with respect to the migrating eye (upper) varies between genera. Point of insertion also varies between ocular and blind side.
11) **Scale:** Nature and type of scales on body varies between ocular and blind side in species; in the same species it sometimes varies at different regions of the body.
12) **Squamation on dorsal and finrays:** Scales may be present/ absent on finrays on ocular and blind side.

Conclusion: Fish length measurements are important for resource assessment and management (Petrell et al., 1997; Harvey et al., 2001a, 2002b; Cadiou et al., 2004), including evaluation of population age structure and biomass for harvest regulations and habitat protection and particularly useful when methods to obtain age or weight are impractical as part of a sampling program (Karpov et al.,1995). Though details of cynoglossid taxonomy is available in plenty, detailed literature on the psettodid morphomeristic taxonomy is lacking. Morpho-meristics of soles is similar to cynoglossids with modification in dorsal fin ray origin position and structural differences on the blind side below the eye. Counts of pectoral and pelvic fin rays which are generally taken only on dorsal side or eyed side of body in case of bilaterally fishes are taken on both sides in the cases of these flatfishes. Since studies on Indian sole fishes is lacking, morphomeristic detailing is also less. A comparative statement of the morphomeristic characters across species along with a compilation of meristic data of previous studies along with the present study can give a bird's eye view of all information as well as the range in different localities studied. This will help easier identification of species. Studies
of morphological variation among populations continue to have an important role to play in stock identification, despite the advent of biochemical and molecular genetic techniques which accumulate neutral genetic differences between groups. (Swain and Foote, 1999). Hence methods in classical taxonomy are to be given more importance and stress in such taxonomic studies. A document on the morphometrics is very important in identification of resources and hence in the documentation of biodiversity. Hence it is important that a consolidated list of the morphomeristic characters of the psettodids, cynoglossids and soles is prepared for future researchers in this area.

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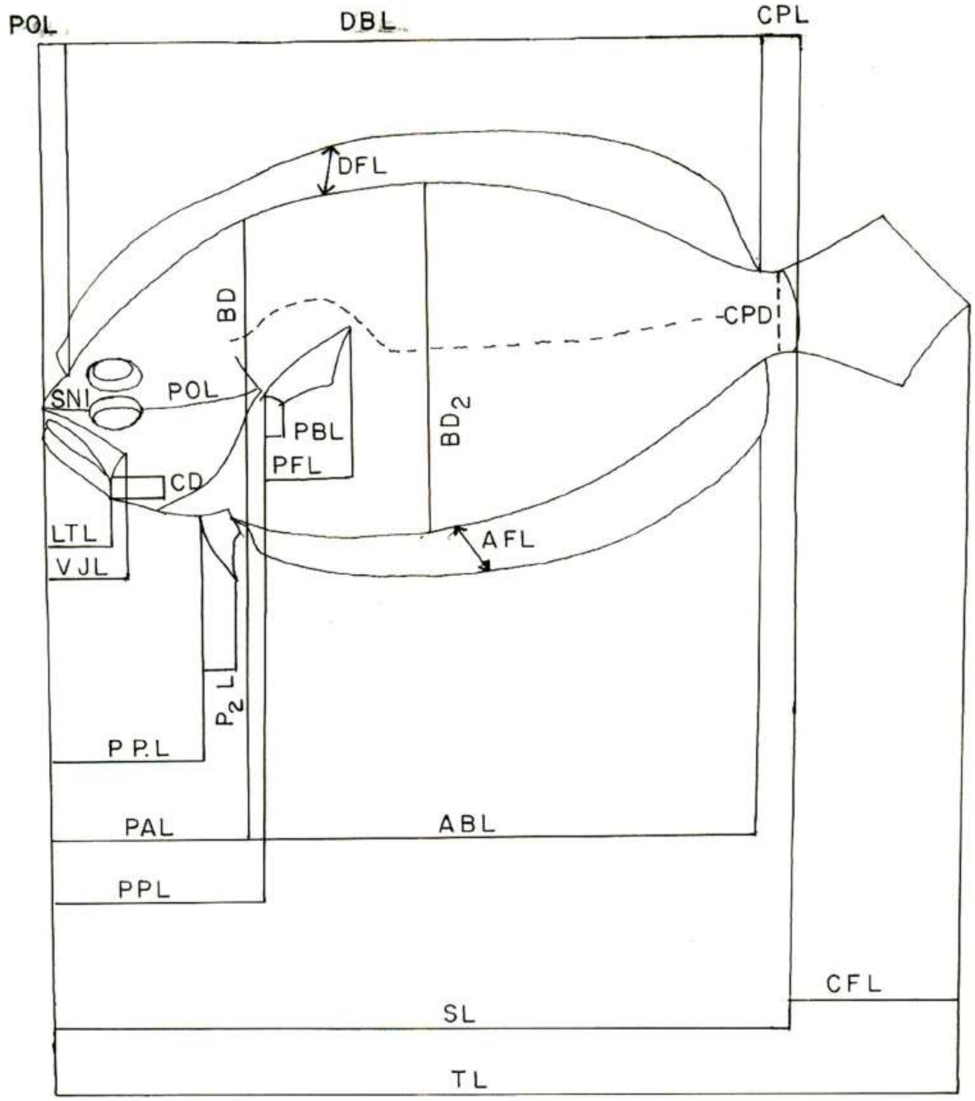

Fig.1 Morphometric measurements on ocular side of Flounder and Halibuts

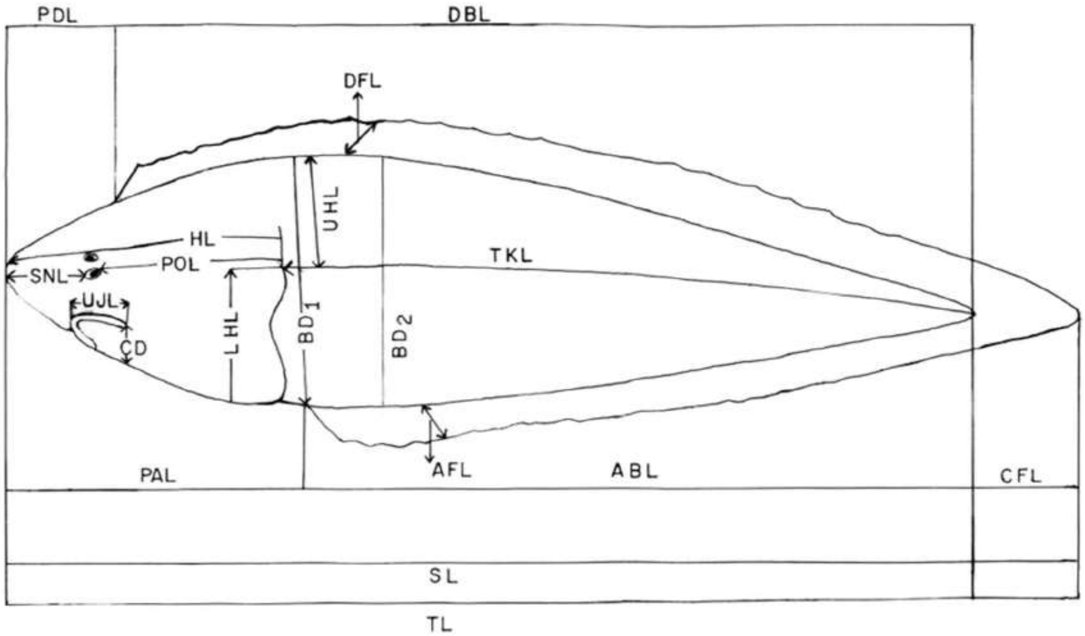

Fig.2 Morphometric measurements on ocular side of Cynoglossids

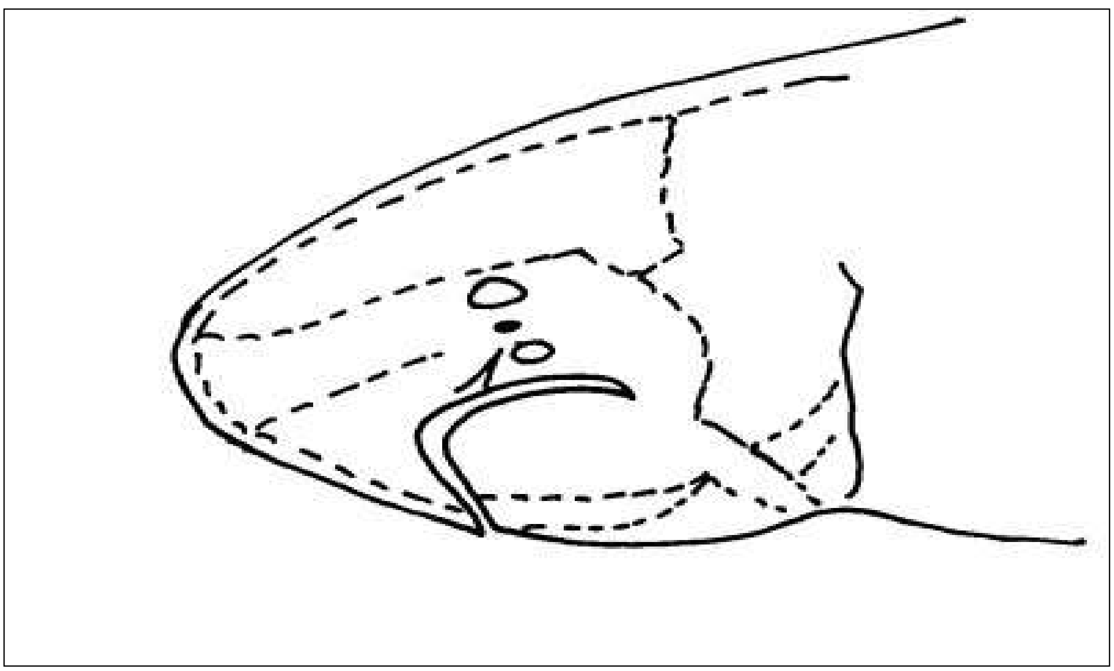

Fig.3 Lateral line pattern on head of Cynoglossid fishes