Training Manual

NAHEP, ICAR-CIFE sponsored

Skill Development Program on

Application of fish taxonomy and tools for identification of fishes

20-26 November 2019

ICAR- Central Institute of Fisheries Education

(University under Sec. 3 of UGC Act),

Indian Council of Agricultural Research

Mumbai 400 061

www.cife.edu.in

Training Manual

Skill Development Programme
on
Application of fish taxonomy and tools for identification of fishes

20 - 26th November 2019

Coordinators

Dr. Shashi Bhushan

Mr. Karan K. Ramteke

Co-Coordinators

Mrs. Layana P.

Dr. Pawan Kumar

ICAR- Central Institute of Fisheries Education

(University under Sec. 3 of UGC Act),

Panch Marg, Off. Yari Road,

Versova, Andheri (west),

Mumbai 400061.

Training Manual
Skill Development Programme On
"Application of fish taxonomy and tools for identification of fishes"
ICAR- Central Institute of Fisheries Education, Mumbai
20-26 November, 2019

Publisher : **Dr. Gopal Krishna**
Director, ICAR- Central Institute of Fisheries Education
Panch Marg, Off. Yari Road, Versova,
Andheri (west), Mumbai- 400061

Edited by **Dr. Shashi Bhushan**
Mr. Karan K. Ramteke
Mrs. Layana P.

Compiled by **Dr. Pawan Kumar**
Mr. Vikas Pathak

Cover Design : **Mr. D. Bhoomaiah**

This manual has been prepared as a reference material for the NAHEP, ICAR-CIFE funded Skill Development Programme on ""Application of fish taxonomy and tools for identification of fishes" during 20-26 November 2019

भारतीय कृषि अनुसंधान परिषद
भा.कृ.अनु.प. - केन्द्रीय मात्स्यिकी शिक्षा संस्थान, मुंबई
(वि. अ. आ. अधिनियम धारा-3 के अंतर्गत विश्वविद्यालय)
ICAR - CENTRAL INSTITUTE OF FISHERIES EDUCATION
(University under Sec.3 of UGC Act)
कृषि एवं किसान कल्याण मंत्रालय, भारत सरकार, Ministry of Agriculture and Farmers Welfare, Govt, of India

डा. गोपाल कृष्णा
निदेशक एवं कुलपति
Dr. Gopal Krishna
Director & Vice Chancellor

FOREWORD

Human beings are always curious about nature and its components. Fish has existed on the Earth for more than 500 million years. More than 60,000 species of the living vertebrates exist on the earth, of which more than half (32,000 species) belongs to the fishes. Tasks of taxonomy basically involve discovering, describing and naming of new species, constructing relatedness and recovering the evolution tree of recorded items. Generally people see taxonomy as the very tedious and complicated, if anyone is interested in nature and the biodiversity, they can master the art of taxonomy. The key to success is to clearly understand the different parts of the bodies of an organism. The interesting things about taxonomy is, view of the researchers vary with the experience, so the classification of the organisms differs as well. The whole conservation and management measures for any fish and fisheries are totally dependent upon the correct identification of the fish species. Every fish population has different biological characteristics, if the species is misidentified or misreported the conservation and management measures applied for any fisheries will go wrong. In our country researchers involved in the taxonomy field are very few and scattered. In this backdrop, Skill Development Programme on "Application of fish taxonomy and tools for identification of fishes" from 20-26 November 2019 organized by the ICAR- Central Institute of Fisheries Education under the aegis of National Agriculture Higher Education Project (NAHEP) with the objective of imparting training on important aspects of fish taxonomical tools for the researchers mainly Master and Ph.D. students from different part of the country to create interest for the taxonomy in these young minds. I compliment the Course Co-coordinators for conceptualizing and organizing the skill development Programme. I am sure that the Programme would be beneficial to the participants in acquiring adequate skills in one of the critical aspects of fish taxonomy so they can utilize the knowledge for the further research and see taxonomy as the important aspects of the science.

(Gopal Krishna)

पंच मार्ग, ऑफ यारी रोड, वरसोवा, अंधेरी (प.) मुंबई - 400 061. भारत
Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061.
कार्यालय / Office : 022-26363404 / 26374306 Fax : 022-26361573
Website : http:// www.cife.edu.in
ई-पत्र / Email : director@cife.edu.in / gopalkrishna@cife.edu.in

Content

Introduction to Taxonomy

Shashi Bhushan, B. B. Nayak and Karan K. Ramteke

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Our planet harbors enormous diversity of living world. All the individuals of a sexually reproducing population are unique (non-identical). This unique nature is the source of enormous diversity. Here taxonomy plays very important role in their identification. Taxonomy defined as theory and practice of classifying organism, is the oldest of biological disciplines (Mayr, 1982, Mayr and Ashlock, 1991). Taxonomy is the part of systematics dealing with the theory and practices of describing diversity and erecting classification (Nelson, 2006). The word taxonomy has been derived from the Greek words – –taxis" means –order" and –nomos" means –law" or –science" which is used to classifying living things according to their shared features. Obviously, taxonomy deals with the ways in which the organisms are grouped together in the world.

Carl Linneas formalized biological classification with his system of binomial nomenclature that assigns each organism a genus and a species name. One of the earliest published works on fish taxonomy is –Systema Ichthyologiae" (1801) by Bloch and Schneider in which 1519 fishes were listed. Cuvier and Valenciennes (1836) provided a great debut to their study of fish taxonomy by publishing the book –Historie Naturelle des Poissons". –Catalogue of Fishes in the British Museum" by Gunther (1838) is one of the greatest works of that time. Berg (1947) classified fishes both recent and fossil while Munro (1995) described marine and freshwater fishes of Ceylon.

Taxonomy makes diversity accessible to other biological disciplines and must remain the basis (Wilson, 2000). In addition to that understanding α-taxonomy is imperative to resource management and biodiversity conservation (Kottelat, 1995). Fishes are endowed with fascinating diversity of more than 32800 species (Froese and Pauly, 2014) making their identification cumbersome. Other reasons making this job more challenging are morphological switch across sexes, developmental stages and geographic ranges. Biodiversity for numerous and obvious reason is in state of crisis. One of the immediate concerns would be the rapid documentation of biodiversity before it is lost. Workers across the globe are endeavoring hard for documenting and assessing biodiversity which requires rapid and reliable taxonomic expertise at the field level. This very practical bottleneck has led to an era of integrative taxonomy including all available data be it morphological, behavioural, bio-geographical, ecological or molecular.

Every year thousands of new species are discovered. In 2016, there have been 183 new species described. In 2015, there were 422 new species added. Total number of fish species described from years 1997 through 2016 was 7401(Eschemyer and Fong, 2016).

In India, the pioneer work on fish taxonomy was publishes –An account of the fishes found in the river Ganges and its branches" Hamilton (1822) which clarified ambiguity in identifying the fishes from regional names. After the book by Hamilton, the milestones in Indian ichthyology was –The Fauna of British India including Ceylon and Burma" by Day (1889).

Advances in Indian fish taxonomy took place during twentieth century with publications from Chaudhuri (1912, 1916), Raj (1916, 1941) and Hora and his coworkers (1920 to1950) based on collections made from India and the neighbouring countries. Most of these works pertain to freshwater fishes. Misra (1962) published "An aid to identification of the common commercial fishes of India and Pakistan" and _The fauna of India and Adjacent countries (Pisces) in 1976. Jones and Kumaran (1980) described over 600 species of fishes from Lakshadweep waters.

Talwar and Kacker (1984) came with a comprehensive description of the marine fishes of India, having commercial Importance. Among recent taxonomic works, the commendable one is of Talwar and Jhingran (1992), covering descriptions of a total of 930 species of inland fishes of India.

Talwar and Kacker (1984) described commercial food fishes of India based on morphological characters. Rajan (2003) described field guide to marine food fishes of Andaman and Nicobar Islands.

Comparative study

Taxonomy Vs Systematics

Mayr (1969) defined taxonomy as –The theory and practice of classifying organisms" where defined systematics as –The science of the diversity of organisms". The systematics is a –science" while taxonomy is –theory and practice". As per the Global Taxonomy Initiative, the taxonomy is the classification of life, so it concentrated on describing species, measures their genetic variability, as well as their relationships with each other.

In 2006, Enghoff & Seberg proposed a broader definition for taxonomy. According to them, taxonomy exists with seven types of activities:

1. Naming and description of taxa (families, genera, species etc., synonymisations, etc.) (α-taxonomy).
2. Classification of taxa (based on phylogenetic analyses) (part of β- taxonomy).
3. Comparison of taxa, with relationship studies (phylogeny) (part of β - taxonomy).

4. Study of (genetic) diversification within species (γ -taxonomy)

5. Making of tools for species identification

6. Identification of specimens (using the tools).

7. Describing of taxa in specific ecosystems (using the tools for identification)

This _Taxonomy of taxonomy' is an enlargement and broadening of a long-existing division of taxonomy into-

α-taxonomy: Descriptive taxonomy

β-taxonomy: Classification

γ-taxonomy: Study of intra-specific diversification

Morphological taxonomy v/s Molecular taxonomy

Morphological taxonomy

Morphological taxonomy is the theory and practice of classifying organisms based on the study of the form and structure of organisms and their unique structural features.

Advantages

Applicability to museum specimens:

It is one of the best advantages of morphological taxonomy over molecular taxonomy due to more applicability in an extensive collection of preserved museums samples, while in general for molecular taxonomy fresh/freeze samples, alcohol or cryopreserved samples required.

In some cases, holotype of a species or type series available in the museum and there is prevention on a collection of another specimen from wild due to the rarity of species. So, for taxonomical study, only one option left out is morphological study.

Applicability to fossil species:

In general, for the paleontological study best and most widely employed method is a morphological investigation which has been preferred due to less applicability of molecular characters to reveal the relationship.

Cost effective: The morphological data can be collected with less expenditure on equipment and supply.

Molecular taxonomy

The theory and practice of classifying organisms on the basis of genetic makeup and their sequence.

Advantages

Size of datasets:

One of the most important advantages of molecular data is a definite size of data set. Because the genetic information of a fish or any organism encoded in DNA and only a part of

this sequence information is examined which reveal the largest possible set of traits for systematic analysis.

The extent of non-heritable variation:

For the phylogenetic analysis, the comparative data is more useful and the considered traits for phylogenetic reconstruction must reveal heritable variation. As per the previous study, it was confirmed that biomolecular data are less influenced by the environmental factor than the morphological data.

Apart from the above-mentioned advantages; some more advantages also pointed out below –

- Through the molecular approaches, ambiguity among the cryptic species can be resolved easily.
- Identification of species at different life stages can be easily done through the molecular approach which is so difficult in the morphological approach, due metamorphosis in life stages.
- even sibling species which have sexual dimorphism can be easily identified through molecular studies
- Through the molecular approaches, species that use camouflage or mimicry for their defense can be identified without any ambiguity.
- For the purpose of molecular taxonomic studies, a smaller number of samples are required

Different methods are utilized in the taxonomic study of fishes.

Classical morphometric measure are based on measurement of various length.

Traditional morphometric analysis

Traditional morphometrics analyzes lengths, widths, areas, angles, and ratios (Marcus 1990).

Elliptic Fourier Analysis (EFA)

Elliptic Fourier analysis can describe any type of closed contour and were effectively applied to evaluate various biological shapes in animals (Ferson *et al.,* 1985, White *et al*., 1988, Diaz *et al.,* 1989, Laurie et al., 1997, Iwata *et al.,* 1998 2000 2002, Yoshioka *et al*., 2004). The elliptic Fourier analysis to is powerful tool for outline analysis and comparing shapes at different taxonomic levels. (Monti *et al*., 2001, Andrade *et al*., 2008, 2010)

Truss analysis

More recent advances have been facilitated by image processing techniques, more comprehensive and precise data collection, more efficient quantification of shape, and new

analytical tools, landmark based techniques of geometric morphometrics (Bookstein, 1991; Rohlf, 1990). These techniques pose no restrictions on the directions of variation and localization of changes in shape; furthermore, they are very effective in capturing information about the shape of an organism (Cavalcanti *et al.,* 1999). Image analysis systems played a major role in the development of morphometric techniques, boosting the utility of morphometric research in fish identification (Cadrin & Friedland, 1999). Morphometry based on truss network data has been used for species discrimination (Palma & Andrade, 2002), ontogeny (Hard *et al.,* 1999) and functional morphology (Dean *et al.,* 2006).

Scales study

The skin of most of fishes are covered by scales. Scales vary in shape, size and structure. Fish scales are part of the fish's integumentary system, and developed from the mesoderm layer, which differentiate them from reptile scales (*Sharpe, 2001)*. The placoid scales of cartilaginous fishes are also dermal in origin.

Morphological characters of many species of fishes including flatheads play very vital role to identify or differentiate them. Hence, it is difficult to determine the exact taxonomic status of species. As fish scales exhibit great variations in their sizes, shapes, structures and arrangements in the different body parts of individual fish (Ikoma *et al*., 2003; Kardong, 2008), there is considerable variation in scale shape and size even between different areas of the same individual fish, scale outline is not always the best indicator for identification (Casteel, 1972; Chikuni, 1968). However, Delamater and Courtenay (1974), Johal and Dua (1994), Helfman *et al*. (1997), Esmaeili *et al*. (2007) and Jawad and Al- Jufaili (2007) have proved that scale characters can be used as valuable tools in the identification of fish up to the genus or species level and its phylogeny. Maitland (2004) provided a key based on scale morphology of the fish families found in freshwater habitats of Britain and Ireland. Several fishery workers have used different scale characteristics for fish identification (Agassiz, 1833-34).

Otolith

Otolith is small stony structure mainly made up of calcium carbonate (CaCO3) which is mostly in the form of aragonite located in inner ear cavity of the teleost fish. They grow continuously, according to an accretionnary process and the accretionary deposit is influenced both by environmental conditions and physiological parameters. These structures act as a balancing and hearing organ (Campana, 1999; Campana and Thorrold, 2001). The otoliths are three dimensional structure but they do not grow at the same rate in all dimensions, (Campana and Thorrold, 2001) Growth of otolith follows an allometric increase

in dimensions (Chilton and Beamish, 1982). Also, shape and size vary considerably among species (C ampana and Thorrold, 2001) Hence morphological characteristics of fish otoliths are highly variable among the species, ranging from the simple disc shape of some flatfish (Pleuronectidae) to the irregular shape of other fish such as redfish (*Sebastes* sp.)

The 10 % of the otolith contain minor and trace elements within the aragonite matrix of otolith that are derived from the surrounding water of fish habitat. These impurities shows water chemistry, as well as fish's metabolism (Telmer, 2004; Campana and Thorrold, 2001).

Barcoding

DNA barcoding is a reliable taxonomic tools for confirmation of species. Although the DNA sequences of related species are generally very similar, still they exhibit difference in the sequence. The part of the DNA sequence are specific for species to species or for a particular organism and forms a unique ahnd specific barcode of DNA. DNA barcodes allow non-experts also to identify species – even from damaged, or industrially processed material. Short DNA barcodes, near about 700 nucleotides in length, can be quickly and unambiguously analyze from thousands of specimens by computer programs.

To meet these needs, a group of scientists joined forces in 2005 to launch the Fish Barcode of Life (FISH-BOL), Ward *et al*. (2009). The current FISH-BOL issue of Mitochondrial DNA provides a 5-year progress report (Becker *et al.* 2011) on the campaign and updated –Collaborators' Protocol" (Steinke and Hanner, 2011) to provide its continued growth and success. The implementation of standards is attributed to success of barcoding program (Teletchea, 2010) and at the end of this program, the new protocol aims to refine and further advance FISH-BOL best practices for the users.

Reference:

Mayr, E., 1982. *The growth of biological thought: Diversity, evolution, and inheritance*, Harvard University Press. 974p.

Mayr, E. and Ashlock, P. D., 1991. *Princples of systematic zoology,second edition,* New York, McGraw Hill, 475p.

Nelson, J.S., 2006. *Fishes of the World.* John Wiley and Sons, New York. 3rd edition. 600pp.

Bloch M. E. and Schneider, S. G., 1801. *Systema Ichthyologiae Iconibus,*valium 2 Berlin.

Cuvier, G. and Valenciennes, A., 1836. *Histoire Naturelles des Poissons.* 22 volumes.

Gunther, A., 1838. *Catalogue of fishes in the British museum.* British Natural History Museum, London. Vol I – VIII.

Berg, L. S., 1947. Classification of Fishes: both recent and fossils. *Acad. Sci*. U.S.S.R, 5(2): 86-517.

Munro, I. S. R., 1955. *The marine and freshwater fishes of Ceylon*. Halstead press, Sydney. 103 225pp.

Wilson, M., 2000. Loss of taxonomists is a threat to pest control. *Nature,* 407**:**559-559.

Kottelat, M., 1995. Systematic studies and biodiversity: the need for a pragmatic approach. *J. Nat. Hist.,* 29**:**565-569.

Froese, R. and Pauly, D. (Eds.), 2014. Fishbase. World wide web electronic publication. www.fishbase.org. ver 2014.

Hamilton, F., 1822. *An Account of the Fishes Found in the River Ganges and Its Branches:* Edinberg and London, 405p.

Day, F., 1889. *The fauna of British India, Ceylon and Burma,* London, Taylor and Francis, 548p.

Chaudhuri, B., 1912. Descriptions of some new species of freshwater fishes from north India. *Rec. Indian Mus.,* 7**:**437-444.

Chaudhuri, B., 1916. Fauna of the Chilka Lake. Fish. Part I. *Rec. Indian Mus.,* 5**:**403-439.

Raj, B. S., 1916. Notes on the freshwater fish of Madras. *Rec. Indian Mus.,* 12**:**249-294.

Raj, B. S., 1941. Two new cyprinid fishes from Travancore, South India, with remarks on Barbus (Puntius) micropogon. *Rec. Indian Mus.,* 43**:**375-386.

Misra, K. S., 1976. *The Fauna of India and Adjacent Countries. Pisces Teleostomi: Cypriniformes and siluriformes* (Vol.3). ZSI, Kolkata, 367p.

Misra, K.S., 1962. *An aid to the identification of the common commercial fishes of India and Pakistan*, ZSI, Kolkata, 320p.

Jones, S. and Kumaran, M., 1980. *Fishes of the Laccadive Archipelago,* Cochin, Mathrubhumi press. 766p.

Talwar, P. K. and Kacker, R., 1984. *Commercial sea fishes of India*, ZSI, Kolkata, 997p.

Talwar, P. K. and Jhingran, A. G., 1992. *Inland fishes of India and adjacent countries,* (Vols. 12). Balkema, 1158p.

Rajan, P. T., 2003. *A field guide to the Marine food fishes of Andaman and Nicobar islands*: ZSI, 260pp.

Mayr, E., 1969. *The growth of biological thought: Diversity, evolution, and inheritance*, Harvard University Press. 974p.

Ferson S., F.J. Rohlf, R.K. Koehn, 1985. Measuring shape variation of two-dimensional outlines. *Syst. Zool*., 34: 59-68.

White R., H.C. Rentice, T. Verwist, 1988. Automated image acquisition and morphometric description. *Can. J. Bot*. 66: 450-459.

Diaz G., A. Zuccarelli, I. Pelligra, A. Ghiani, 1989. Elliptic Fourier analysis of cell and nuclear shapes.Compar. *Biomed. Res*., 22: 405-414.

Laurie C.C., J.R. True, J. Liu, J.M. Mercer, 1997. An introgression analysis of quantitative trait loci that contribute to a morphological difference between Drosophila simulans and D. mauritiana. *Genetics,*145: 339-348.

Iwata H., S Niikura, S. Matsuura, Y. Takano and Ukai Y., 1998. Evaluation of variation of root shape of Japanese radish (Raphanus sativus L.) based on image analysis using elliptic fourier descriptors. *Euphytica,* 102: 143-149.

Iwata H, S. Niikura, S. Matsuura, Y. Takano, Ukai Y., 2000. Diallel analysis of root shape of Japanese radish (Raphanus sativus L.) based on elliptic Fourier descriptors. *Breed. Sci*. 50: 73-80.

Iwata H, Y Ukai. 2002. SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered*., 93: 384-385.

Iwata, H. and Ukai Y., 2002. SHAPE: A computer program package for quantitative evaluation of biological shapes based on elliptic fourier descriptors. *J. Hered.,* 93: 384-385.

Andrade I.M., S.J. Mayo, D. Kirkup, C. Van den Berg, 2008. Comparative morphology of populations of Monstera (Araceae) from natural forest fragments in northeast Brazil using elliptic Fourier analysis of leaf outlines. *Kew Bull.,* 63:193-211.

Andrade I.M, S.J. Mayo, D. Kirkup, C. Van den Berg, 2010. Elliptic Fourier analysis of leaf outline shape in forest fragment populations of Anthurium sinuatum and A. pentaphyllum (Araceae) from northeast Brazil. *Kew Bull*. 65:1-18.

Marcus, L. F., 1990. Chapter 4. Traditional morphometrics. In Proceedings of the Michigan Morphometric Workshop. Special Publication No. 2. *F. J. Rohlf* and *F. L. Bookstein*. Ann.

Sharpe, P. T., 2001. "Fish scale development: Hair today, teeth and scales yesterday?". Current Biology, 11 (18): R751–R752.

Ikoma, T., Kobayasi, H., Tanaka, J., Walsh, D. and Mann, S., 2003. Physical properties of type I collagen extracted from the fish scales of *Pagrus major* and

Oreochromis niloticus. *International Journal of Biological Macromolecules,* 32(3-5): 199-204.

Kardong, K., 2008. *Vertebrates: Comparative anatomy, function, evolution*. 2nd edition. McGraw Hill.

Campana S. E., 1999: Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser*., 188: 263–297.

Campana S. E., Thorrold S. R., 2001: Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci*., 58: 30–38.

Steinke D and Hanner R., 2011. The FISH-BOL collaborators protocol. *Mitochondrial DNA,* 22 (Supp. 1):10–14.

Teletchea F. 2010. After 7 years and 1000 citations: Comparative assessment of the DNA barcoding and the DNA taxonomy proposals for taxonomists and non-taxonomists. *Mitochondrial DNA* 21:206–226.

The evolution of the fishes

Shashi Bhushan, Vikas Pathak and Karan K. Ramteke

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Fish are a major part of the marine ecosystem and this has been the case for hundreds of millions of years. The origin of fish can be traced as far back as the Cambrian (541 million year ago) explosion over five hundred million years ago however it's not until the Devonian period, 100 million years later (419. Mya) the fish reaching their maximum diversity after rise and fall of many of major groups (fig. 1).

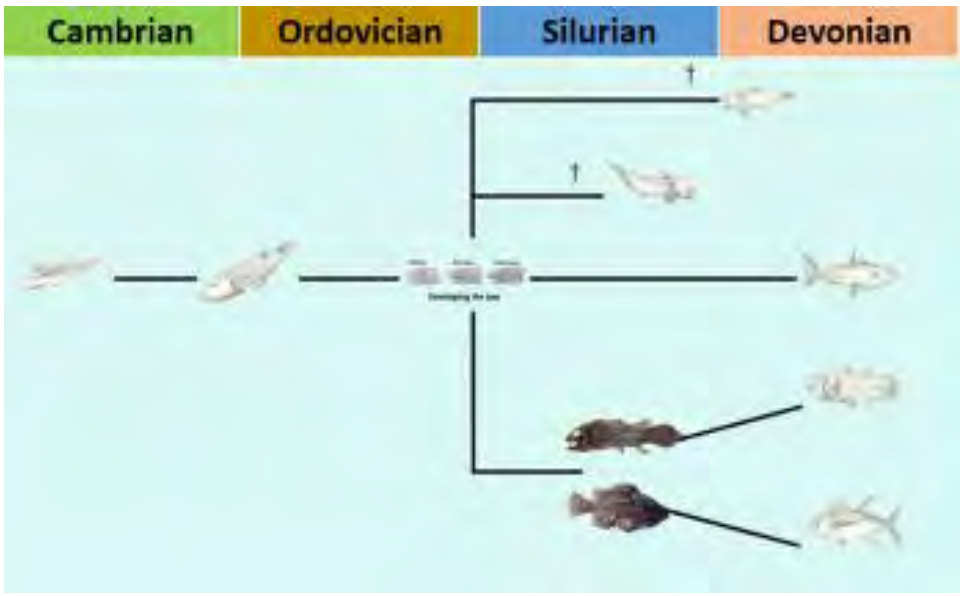


Fig. 1 Age of fish through Time

Cambrian

The origin of fish can be traced as far back as the Cambrian explosion 'is early fish were small, jawless and filter feeders with relatively simple bodies (fig. 2). Up to the end of the Cambrian the simple body plan starting to become more complex as Ostracoderms (cephalaspis) are first seen in the late Cambrian they differed from early fishes as they use their gills exclusively for respiration and not feeding they also had armored bodies which deterred their predators and this meant that they were most likely slow swimming bottom dwellers, also lacked jaws which limit them to feeding on smaller simple foods which they consumed through suction (fig. 3)..

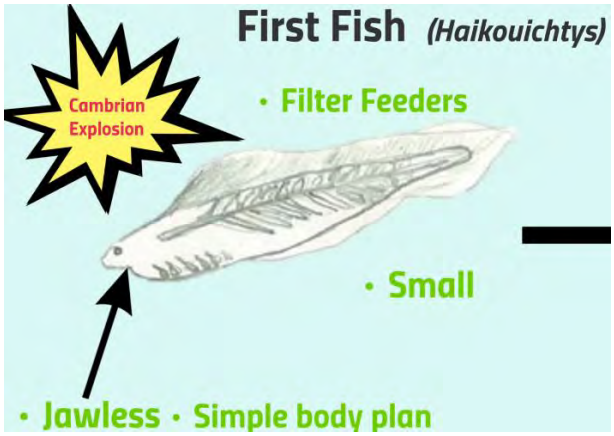


Fig. 2. Early Cambrian fishes

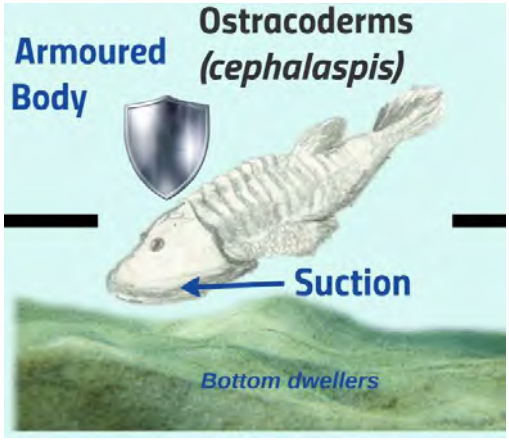


fig. 3. Late Cambrian fishes

Ordovician

The next major development seen in the evolution of fish is the development of the jaw, this first occurred in the late Ordovician (445.2 Mya) and can be seen as perhaps the single most profound and radical evolutionary steps in vertebrate history following its development. The diversity of fish increased dramatically the jaw likely developed as a result of the moving a modification of gill arches in jawless fish into the mess forming a rugged hinged structure (fig. 4), the first jaw a hinged jaw allowed fish to catch and hit to much larger food items, this innovation arrived fish to become large predators for the first time in history.

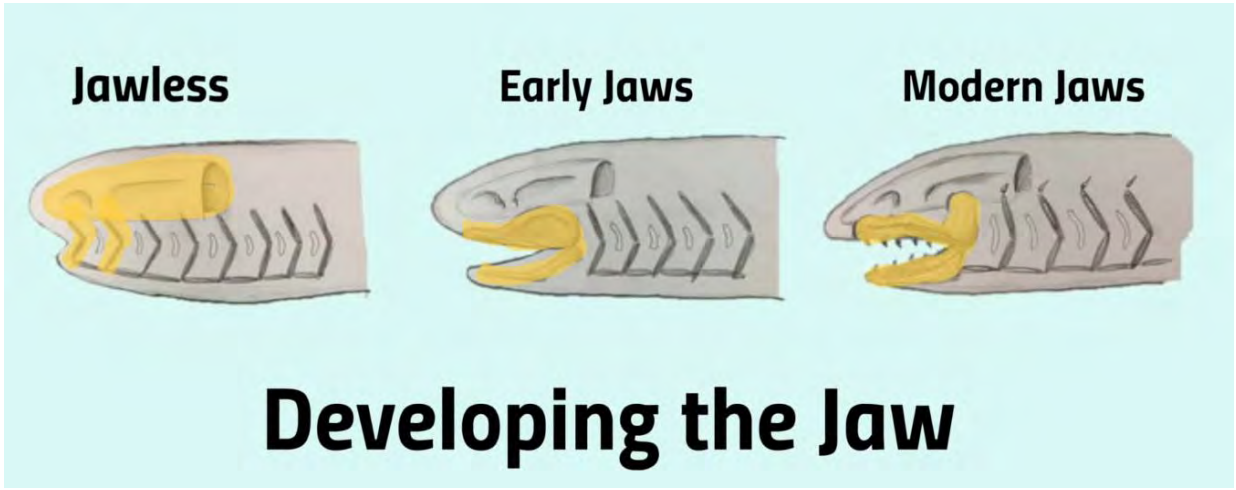


Fig. 4. Hinged structure of jaws

Silurian

Among the first jawed fishes having armor on the head and neck although this class of fish is first seen in the late Silurian (fig. 5). It is not until the Devonian as these fishes undergo major biodiversity explosion. The placoderm (Dunkleosteus) become the most

abundant form of vertebrate life during this period and dominate the Devonian with the innovation of jaws and some placoderm became planets first vertebrate super predators. The placoderm spiny sharks (Acanthodii) were among the first jawed fish. This bizarre lineage are always in early Silurian, the name of spiny shark is somewhat of a misnomer despite their similar appearances, they are in fact not a true sharks, get their name from the bony spines which supported their fins and their shark like body shape (fig. 6).

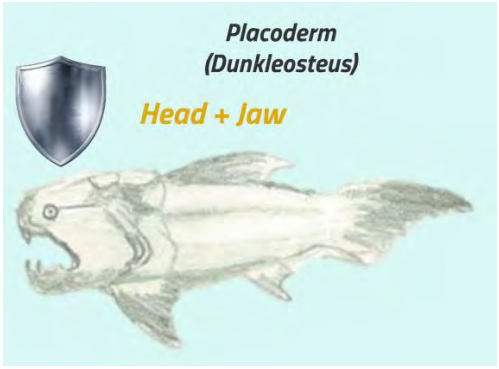


Fig. 5. Late Silurian fish

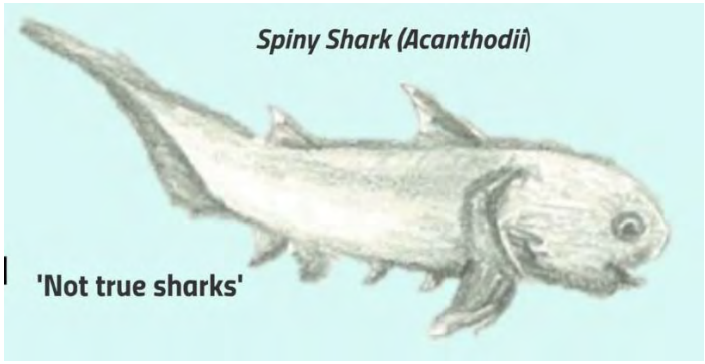


fig. 6. Spiny shark

Devonian

During the Devonian period many species of spiny shark moved from marine to freshwater environments, this transition likely occurred to avoid the highly competitive marine environment, in this new environment the spiny sharks thrive as a small Swift predator.

The chondrichthyes is a large class of fish which includes sharks, skates, rays and chimeras. The members are characterized by a skeleton made of cartilage instead of bone, toothed jaws and a body covered in toothless scales. The first cartilaginous fish are seen in the late Silurian in the form of early sharks but in the Devonian sharks growing relatively large and taking up their traditional role of predators (fig. 7).

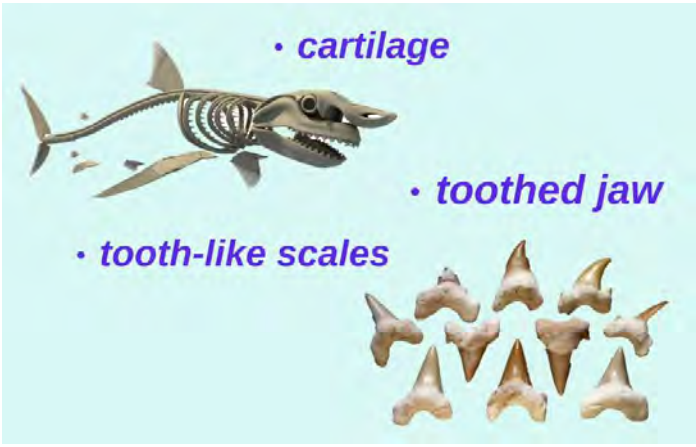


Fig. 7. Devonian sharks

Bony fish get their name from the rigid skeleton which constructed totally out of bone, the huge lineage first appears in the fossil record in the late Silurian remains becoming more common throughout the Devonian. The osteichthyes can be divided into two main groups the ray-finned and lob finfish.

Ray-finned fish

The ray-finned fish differ from their lob - fined fish counterparts on account of the construction of their fins as the name suggests ray-finned fish have fins constructed from pin bony rays which are connected with the fine web of skin, these fins are powered by muscles inside the body (fig. 8). The fossil record suggests that diversity of this class is moderate in the Devonian.

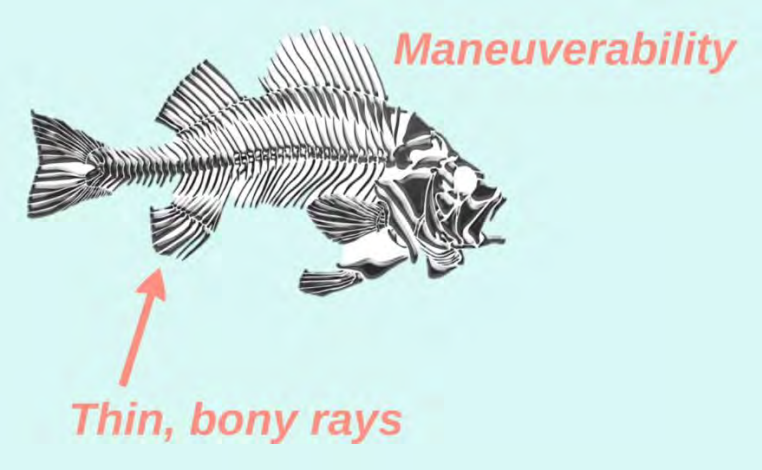


Fig. 8. Ray-finned fish

Lobe-finned fish

This class is characterized by fleshy fins with the robust internal skeleton and musculature (fig. 9). The lobe fin fish display massive diversity during the Devonian period, they were especially successful in freshwater environments where they received less competition. In the freshwater environments lobe-fin fishes tribe.

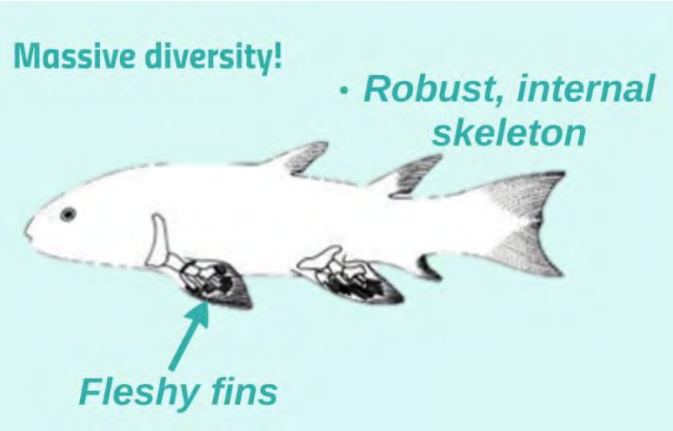


Fig. 9. Lobe-finned fish

In the late devonian a pioneering group of lobed fin fish made the transition from water to land becoming the first tetrapods, every living amphibians, reptiles, mammals and birds can trace its origins back to this major evolutionary step but all was not meant to be (fig. 10). The end of the Devonian is marked with a mass extinction which claimed the Placoderms and Ostracoderms.

Fig. 10. Origin of Amphibians

Placoderm

Placoderm sharks dominate all aquatic ecosystems as major predators, the body part of the shark has remained largely unchanged since the late Silurian and Devonian this perhaps displays what a successful design of this despite their meager start in the late Silurian and Devonian. The ray fin fishes are perhaps one of the great successes of the evolution of vertebrates following the Devonian, their diversity remained modest until the Mesozoic era, it's at this point that the ray fin fish began to undergo major evolutionary radiation which continues to this day these evolutionary adaptations which have been successful for millions of years be able to adapt the impact of modern day.

Reference

Arratia, G. 1997 Basal teleosts and teleostean phylogeny. Palaeo Ichthyologica 7, 1– 168.

Arratia, G. 2000 Remarkable teleostean fishes from the Late Jurassic of southern Germany and their phylogenetic relationships. Mitt. Mus. Nat.kd., Berl., Geowiss. Reihe 3, 137–179.

Grande, L. & Bemis, W. E. 1998 A comprehensive phylogenetic study of amiid fishes (Amiidae) based on comparative skeletal anatomy. An empirical search for interconnected patterns of natural history. J. Vertebr. Paleontol. 18(Suppl. 1), 1–690.

Graur, D. & Martin, W. 2004 Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. Trends Genet. 20, 80–86.

Quicke, D. L., Taylor, J. & Purvis, A. 2001 Changing the landscape: a new strategy for estimating large phylogenies. Syst. Biol. 50, 60–66.

Regan, C. T. 1923 The skeleton of Lepidosteus, with remarks on the origin and evolution of the lower neopterygian fishes. Proc. Zool. Soc. 1923, 445–461.

Schaeffer, B. 1973 Interrelationships of chondrosteans. In Interrelationships offishes (ed. P. H. Greenwood, R. S. Miles & C. Patterson), pp. 207–226.

London, UK: Academic Press. Schmidt, H. A., Strimmer, K., Vingron, M. & von Haeseler, A. 2002 TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18, 502–504.

Steinke, D., Salzburger, W. & Meyer, A. 2006 Novel relationships among ten fish model species revealed based on a phylogenomic analysis using ESTs. J. Mol. Evol. 62, 772–784.

Swofford, D. L. 1998 Phylogenetic analysis using parsimony (and other methods), v. 4.0. Sunderland, MA: Sinauer Associates.

Taylor, J. S., Van de Peer, Y., Braasch, I. & Meyer, A. 2001 Comparative genomics provides evidence for an ancient genome duplication event in fish. Phil. Trans. R. Soc. B 356, 1661–1679.

Venkatesh, B., Erdmann, M. V. & Brenner, S. 2001 Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. Proc. Natl Acad. Sci. USA 98, 11 382–11 387.

Yamanoue, Y., Miya, M., Inoue, J. G., Matsuura, K. & Nishida, M. 2006 The mitochondrial genome of spotted green pufferfish Tetraodon nigroviridis (Teleostei: Tetraodontiformes) and divergence time estimation among model organisms in fishes. Genes Genet. Syst. 81, 29–39.

Species concept

Vikas Pathak, Shashi Bhushan and Abhijit Mallik

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Introduction

Species is the fundamental unit in biology and the building bricks in biological classification from which concepts of higher and lower groups are developed, the scientific system of naming, kinds of plants and animals revolves around the species level. The term _Species' is Latin word, stand for _Kinds' according to International Code of Botanical Nomenclature (ICBN) which has attempted to clarify the meaning of the word species. _Species are convenient classificatory units defined by trained biologists using all information available'. The word species has a dual connotation in biological science, first- Species is a naturally occurring group of individual organisms that comprises a basic unit of evolution, second- The species is a category with a taxonomic hierarchy governed by various rules of nomenclature. Since ancient time philosophers and naturalists realize that the necessity for a basic unit by which biodiversity on this planet may be described and estimated. Charles Darwin said _Every biologist knows approximately what is meant when we talk about species yet no other taxon has been subjected to such violent controversies as to its definition'.

The word species has different meaning for different taxonomist, Biological species arise by an evolutionary process called as speciation. The term speciation was coined by Cooke in 1906. The basic process of evolution recognizes the existence of two processes namely Anagenesis- phyletic change in the course of time, cladogenesis or speciation- the origin of new species of organisms through separating of pre-existing ones. Species is also considered as one of the main ways by which organisms adapt in order to exploit the diversity of environments available to them.

A number of possible modes of speciation have been suggested by various biologists, Julian Huxley (1887-1975) suggested three types of speciation namely Geographical, Ecological and Genetic. Ernst Mayr (1904-2005) classified the various modes of speciation into the following type's - Geographic speciation, Semi geographic speciation and non-geographic or sympatric speciation- instantaneous and gradual. Swall Wright (1889 – 1988) proposed seven alternative modes of speciation, which are basically overlapping with each other and based on the size of population and the number of ancestral species giving rise to new species. Peter D. Ashlok (1929 – 1989) and Ernst Mayr (1904 – 2005) proposed six possible modes of speciation like Polyploidy, Sympatric, Parapatric, Geographic or allopatric (which has two types) dico Patrick and Perry Patrick and speciation in time.

Development of species concept

Greek philosophers and naturalist like Hippocrates (460 – 370 BC), Plato (427 – 347 BC) and Aristotle (384 – 322) have paid attention to biological classification in which species is the basic unit. Hippocrates described types of animals but there is no indication of any useful classification in his work, Plato believed in essentialism, which is also referred to as the theory of forms and used the term _Eidos' for forms or types; it is of Greek origin and serves to designate any of those primary realities, which came to be known as the forms. Aristotle is a father of biological classification, as far as evolution is concerned, gave the idea of the ladder of life, a series in which organisms could be arranged in the order of increasing complexity and emphasizes that all the attributes of animals such as living, actions, habits and body parts may be taken into consideration in classification, his idea was also a kind of typological or essentialism as far as species is concerned.

Carolus Linnaeus (1707- 1778) was a great taxonomist and sometimes called the _father of taxonomy', adhered to downward classification. The typological definition of species based on the concept of Linnaeus is called the essentialist species concept.

An entirely new concept begun to emerge in the 17th century, John Ray believed in the morphological definition of species and his species characterization also contained the German biological species concept which considers the reproductive relationship to be a principle species criterion, as early as 1760 Joseph Gottlieb Koelreuter (1733 – 1806) mentioned all the individuals which are able to interbreed and produce fertile progeny belonged to the same species. Comte de Buffon (1707 – 1788) in Histoire Naturelle, described everything known in the natural world and believed in organic change but did not provide any mechanism to explain the evolutionary change, Buffon prepared the way for biological species concept using sterility barrier instead of morphological similarities as species criterion.

The biological species concept was clearly formulated by Claude Thomas Alexis Jordan (1814 – 1897), Theodosius Dobzhansky (1900 – 1995) and Ernst Mayr (1904 – 2005), according to mayr, A species is a group of potentially or actually interbreeding natural populations which are reproductively isolated from other such groups, however, Dobzhansky being an evolutionary genetist defined Species as a reproductive community of sexually and cross fertilizing individuals which share in a common gene pool. The biological species concept is the most widely accepted but it has certain difficulties in its application, since biological species concept is applicable to non-dimensional situation. George Gaylord Simpson (1902 – 1984) faced with problem of studying the evolution of species through time, proposed evolutionary species concept in which a species is a lineage (an ancestral -

descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies.

Types of species concepts

Survey of taxonomic literature shows that there are a large number of species concepts which have been proposed from time to time there are more than 20 species concepts which are listed below-

Agamospecies

Asexual lineages, uniparental organisms (apomicts and parthenogens) that cluster together in terms of their genome, maybe secondarily uniparental from biparental ancestors.

Biological species

Mendelian populations of sexually reproducing organisms, interbreeding natural populations isolating from each other, depending upon reproductive isolating mechanisms.

Cladistics species

Set of organisms between speciation events are between speciation and extinction events are a segment of a phylogenetic lineage between nodes

Cohesion species

Evolutionary lineages bounded by cohesion mechanisms that cause reproductive communities particularly genetic exchange and ecological interchangeability

Composite species

All organisms belonging to an internodon and their descendants until a subsequent internodon (an internodon is a set of organisms whose parent-child relations are not split)

Ecological species

A lineage which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range

Evolutionary species

A lineage (ancestral - descendant sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies.

Evolutionary significant unit

A population (or group of populations) that is substantially reproductively isolated from other conspecific population units and represents an important component in the evolutionary legacy of the species.

Genealogical concordance

Population subdivisions concordantly identified by multiple independent genetic units constitute the population units worthy of recognition as phylogenetic taxa.

Genetic species

Group of organisms that may inherent characters from each other, common gene pool, reproductive community that forms a genetic unit.

Genotypic cluster

Clusters of monotypic or politic biological entities, identified using morphology or genetics, forming groups that have few or no intermediates when in contact

Hennigian species

A tokogenetic community that arises when a stem species is dissolved into two new species and ends when it goes extinct or speciates.

Inter noodle species

Organisms are conspecific in virtue of their common membership of a part of a genealogical network between two permanent splitting events or a splitting event and extinction.

Morphological species

Similar to typological species concept of Linnaeus; species are the smallest groups that are consistently and persistently distinct and distinguishable by ordinary means.

Nominalistic species

Only individuals exist and nothing more. Species have no actual existence in nature

Non-dimensional species

Species delimitation in a no - dimensional system (a system without the dimensions of space and time)

Nothospecies

Species formed from the hybridization of two distinct parental species, often by polyplidy

Phonetic species

A cluster of characters that statistically co-vary, a family resemblance concept in which possession of most characters is required for inclusion in a species, but not all

Phylogenetic species

A species is the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and decent.

Recognition species

A species is that most inclusive population of individuals, bi-parental organisms which share a common fertilization system.

Reproductive competition species

The most extensive units in the natural economy such that reproductive competition occurs among their parts.

Successional species

Arbitrary anagenetic stages in morphological forms, mainly in the paleontological records.

Taxonomic species

Species considered by taxonomist to be a member of a kind on the evidence or the assumption that they are as alike as their offspring of hereditary relatives within a few generations.

Four groups of innumerable species concepts

Mayr and Ashlock stated that the innumerable species concepts as discussed above fall into four groups, the first two have mainly historical significance but are still upheld by a few contemporary authors, these groups are-

1. Typological or taxonomic species concept
2. Nominalistic species concept
3. Biological species concept
4. Evolutionary species concept

Taxonomic Species Concept

According to this concept, the species is regarded as an assemblage of individuals with morphological features in common and separable from other such assemblages by correlated morphological discontinuity in a number of features. The individuals of a species show continuous variations, share certain characters and show a distinct discontinuity with individuals belonging to other species with respect to all or some of these characters

Einar Gustaf Du Rietz (1895 – 1967) modified the taxonomic species concept by also incorporating the role of geographic distribution of populations and developed the morpho-geographical species concept. The species was defined as the smallest population that is permanently separated from other population by distinct discontinuity in a series of bio types.

The populations recognized as distinct species and occurring in separate geographical areas are generally stable and remains so even when grown together. There are however, examples of few species pairs which are morphologically quite distinct well adapted to respect to climates but when grown together they readily interbreed and form intermediate fertile hybrids bridging the discontinuity gap between the species examples are *platanus*

orientalis of the mediterranean region and *platanus Occidental* as' of United States such pair of species are known as vicarious species or vicariance and the phenomenon is known as *vicariance arbic arizim*

Morphological and morpho-geographical types of taxonomic species have been widely accepted by taxonomist, however, take into account, the data from genetics, ecology extra but firmly believe that species recognized must be delimited by morphological characters.

Advantages of Taxonomic Species Concept

1. It is useful for general taxonomic purposes especially the field and herbarium identification of plants
2. The concept is widely applied and most species have been recognized using this concept
3. The morphological and geographical features used in the application of this concept can be easily observed in populations
4. Experimental taxonomist who do not recognize this concept apply in cryptic form

Disadvantages of Taxonomic Species Concept

1. it's highly subjective and different sets of characters are used in different groups of organism
2. It requires much experience to practice this concept because only after considerable observation and experience, a taxonomist can decide the characters, which are reliable in a particular taxonomic group
3. The concept does not take into account the genetic relationship between organism

Biological species concept

The biological species concept was first developed by Mayer, defined species as groups of actually are potentially interbreeding natural populations, which are reproductively isolated from other such groups.

Based on the same criteria Verne Edwin Grant (1917 – 2007) defined, species as a community of cross-fertilizing individuals linked together by bonds of mating and reproductively isolated from other species by barriers to meeting.

The recognition of biological species thus involves

a. Interbreeding among populations of the same species
b. Reproductive isolation

The biological species concept is the most held conceptually by systematics. Most practicing workers would believe that the morphological differences used for the species delimitation to indeed reflect similar degrees of interbreeding and reproductive isolation. Hence although the morphological, most workers adhere to the broader conceptual base of the biological species concept.

The utility of the biological species concept has spawned many applications and added perspectives clearly, one of the reasons for its utility is that it deals with the reproductive isolation which is admitted by nearly all the workers to be important in evolutionary theory.

Advantage of Biological Species Concept

a. The objective and at the same criteria is used for all the groups of fishes.
b. It has a scientific basis as the populations showing reproductive isolation do not enter-mix and the morphological differences are maintained even if the species grow in the same area.
c. The concept is based on the analysis of features and does not need experience to put it into practice.

Problems Encountered during Applicability of Biological Species Concept

a. A good majority of plants and lower invertebrate show vegetative reproduction, hence, the concept of reproductive isolation as such cannot be applied
b. The reproductive isolation is commonly verified under experimental conditions, usually under cultivation. It may have no relevance for wild populations.
c. Genetic changes causing morphological differentiation and those cause in reproductive barriers do not always go hand in hand.
d. Fertility sterility is only a theoretical value in allopatric populations
e. Necessary genetic and experimental data are available for only very few species

Ledyard Stebbins combined two concepts and stated that species must consist of system of pollutions that are separated from each other by complete or at least sharp discontinuities in the variation pattern and that these discontinuities must have a genetic basis

Evolutionary species concept

This concept was developed by George Gaylord Simpson (1902- 1984) also stated that Species as a lineage (an ancestral descendent sequence of populations) evolving separately from others and with its own unitary evolutionary role and tendencies,

although maintaining interbreeding among sexually reproducing individuals is an important component in species cohesion. E.O. Wiley defines, An evolutionary species is a single lineage of ancestor - descendant populations which maintains its identity from other such lineages, and which has its own evolutionary tendencies and historical fate.

Mayer has criticized the evolutionary species definition saying that it is the definition of a phyletic lineage, but not of species. it is also applicable to incipient species or isolated populations. Further, it ignores the core of the species problem and tries to delimit species taxa in the time dimension. this concept avoids many of the problems of the biological concept lineage.

Micro species

Term micro species was first suggested by Jordan, micro species are distinct from cryptic species which are morphologically similar but cytologically or physiologically different. C.A. Stace uses the term semi cryptic species for the later

Reference

Buch Lvon, 1825. Uebersicht der flora auf den canarischen Inseln. In: Physicalische Beschreibung der Canarischen Inseln. Berlin, 105–200.

Buffon G, 1749–1804. Histoire naturelle, générale et particulière, 44 vols. Paris.

Cramp S, Simmons KEL, 1977. Handbook of the Birds of Europe, the Middle East and North Africa The Birds of the Western Palearctic, Vol. 1. Oxford: Oxford University Press.

Darwin C, 1859. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. London:

John Murray. Del Hoyo J, Elliott A, Sargatal J, 1992. Handbook of the Birds of the World, Vol. 1. Barcelona: Lynx ediçions.

Dickinson EC, 2003. The Howard and Moore Complete Checklist of Birds of the World, 3rd edn. London: Helm.

Mayr E, 1991. One Long Argument. Charles Darwin and the Genesis of Modern Evolutionary Thought. London: Penguin Press. Mayr E, Ashlock PD, 1991. Principles of Systematic Zoology. New York: McGraw-Hill. Mousson A, 1849. Die Land- und Süsswasser-Mollusken von Java. Zürich.

Ray J, 1686. Historia Generalis Plantarum. London. Rensch B, 1928. Grenzfälle von Rasse und Art. J. Ornithol. 76: 222–231.

Rensch B, 1929. Das Prinzip geographischer Rassenkreise und das Problem der Artbildung. Berlin.

Szalay F, Bock WJ, 1991. Evolutionary theory and systematics: relationships between process and patterns. Z. zool. Syst. Evol.forsch. 29: 1–39.

Wagner M, 1868. Die Darwin'sche Theorie und das Migrationsgesetz der Organismen. Leipzig.

Wallace AR, 1864. The Malayan Papilionidae or swallow-tailed butterflies, as illustrative of the theory of natural selection. In: Wallace AR ed. Contributions to the Theory of Natural Selection. London, 130–200.

Cryptic species

Vikas pathak, Suman Nama, Sri Hari M. and Shashi Bhushan

ICAR - Central Institute of Fisheries Education off- Yari Road Campus, Mumbai

Historically groups of organisms have been assigned species status based on having the same or similar morphology that is all members of a species appear to have similar characteristics when observed by the human eye, this doesn't mean to say that all members of a species are identical however, variation often exists between members of a species. Now a day's species are generally defined by their ability to produce viable offspring, although a number of different species concepts exist from past 300 years. It is known that many species exist which appear to be indistinguishable by their morphology alone or –Two or more species that are erroneously classified (and hidden) under one species name" are known as cryptic species and are found in fungi, animals, plants and other forms of life such as bacteria.

The presence of cryptic species can have a number of consequences including the misidentification of economically and agriculturally detrimental pest species such as *Fusarium oxysporum*. Cryptic species are also important when considering human health for example of the malaria spreading mosquito complex *Anopheles gambiae* in which there are at least seven morphologically identical species. Only certain members of this complex actually transmit malaria. Humans and cities important to be able to identify them by other means for example by differences in their behaviour and their genetics.

The species are not the lowest taxonomic rank that can be assigned to an organism, subspecies exist where groups of organisms are evolutionarily distinct and they're able to interbreed with other closely related groups

For example Previously, *D. auripinnis* having been treated as nothing more than a colourful variation of *D. trimaculatus*. It might seem hard to believe that such drastically different fishes could be confused, but much of this has to do with the isolated distribution of *D. auripinnis* (fig. 3a and 3b). The main trait to look for when identifying this species is the almost solidly yellow caudal, anal and pelvic fins, which, at the most, have a thin, black margin. The Genetic data has highlighted the uniqueness and discriminate these species.

Above species are good example of cryptic species, distinct species have long been proposed based on presence of spot on body (fig. 1) and stream line on lateral-line (fig. 2).

The taxonomic uncertainty needs two levels of biodiversity being underestimated and can negatively impact on conservation efforts. As a result fortunately there a variety of techniques to decide taxonomically rank of organisms. Genetics is one of the most recently adopted tools that can be used in taxonomy.

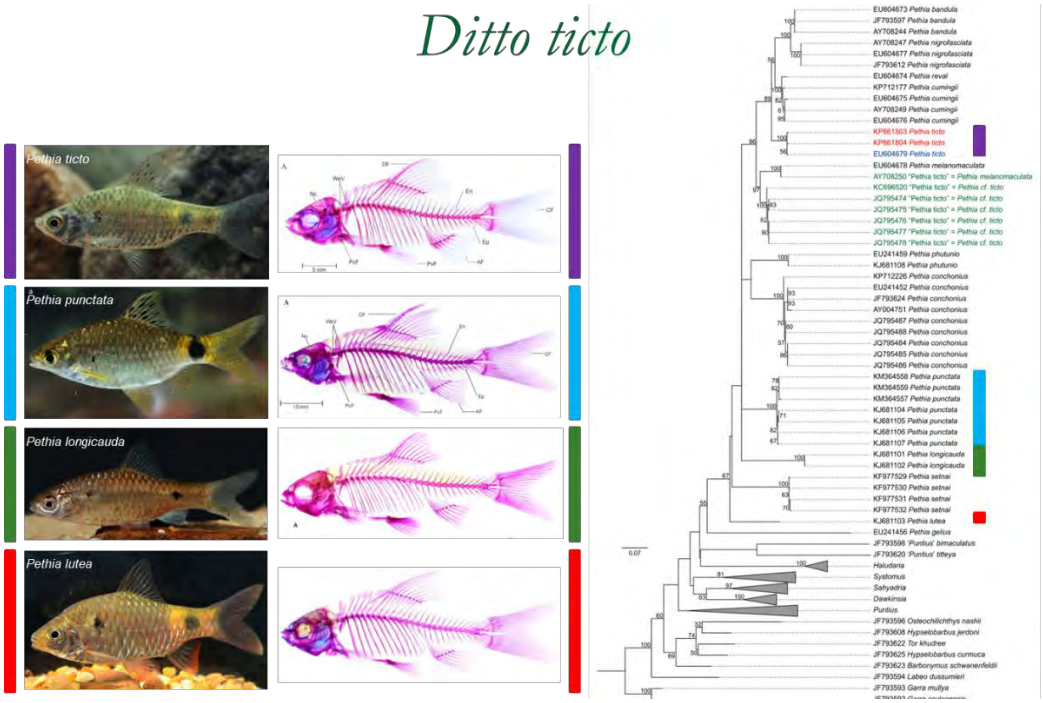


Fig. 1

Chela laubuca

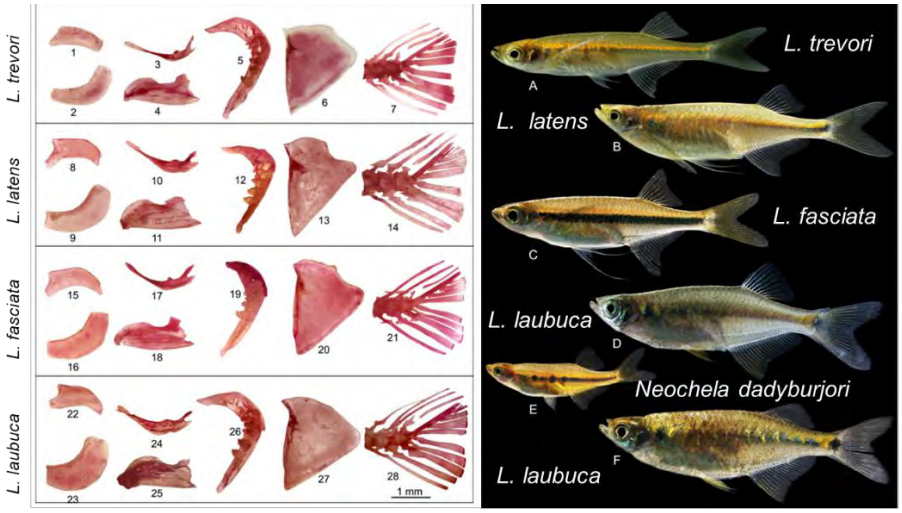


Fig. 2

DNA sequences can be used to infer relationships between specimens and allow to build evolutionary trees that show where the specimens belong to separate species. Databases

such as the barcode of life or bold compiler information such as geographic location and images of specimens along with short DNA sequences. Once this information has been validated and approved then it is made accessible to the public on the bold website and then can be used with other research. The website offers a number of ways to access and search for information including an identification search tool. The main drawback to the barcode of right database is that it limited, to a select few genetic markers, therefore other genetic databases such as Gen-Bank should be used for taxonomic research that requires multiple genetic loci for species delimitation (fig. 3 a, b).

Fig. 3a - *Dascyllus auripinnis* and 3b - *D. trimaculatus*

According to many academics institute the genetics should not be the end-all of taxonomy, and the museums play an important role in the process that has been dubbed as integrated taxonomy. The museum have millions of objects and sometimes a whole series of the same species or the same type of pot so multiple of the same things, in such a way museum is an important resource for researchers because it means that researcher can go in and examine the details of difference. Different individuals of the same species researcher can see the changes with size and sex. The changes might happen because of biogeography, where things have come from.

Other thing that museums have type specimens so if someone goes out and finds a species that's new to science they have to describe this species so that other scientists can go and evaluate their work and that's example which they first found is designated as a type specimen. So the physical object on which scientific name is attached and the best place to have these is in museums because then people can see them and compare with their own findings.

Reference:

Allen, L.G., Bouvier, L.S. & Jensen, R.E. 1992. Abundance, diversity and seasonality of cryptic fishes and their contribution to a temperate reef fish assemblage off Santa Catalina Island, California. Southern California Academy of Sciences, 91(2): 55-69.

Brandl, S.J., Goatley, C.H., Bellwood, D.R. & Tornabene, L. 2018. The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. Biological Reviews, 93(4): 1846-1873.

Friedlander, A.M. & Parrish, D.J. 1998. Habitat characteristics affecting fish assemblages on a Hawaiian coral reef. Journal of Experimental Marine Ecology, 224(1): 1-30.

Philippe Borsa, Andrianus Sembiring, Cécile Fauvelot and Wei-Jen Chen, 2014. Resurrection of Indian Ocean humbug damselfish, Dascyllus abudafur (Forsskål) from synonymy with its Pacific Ocean sibling, Dascyllus aruanus (L.), Comptes Rendus Biologies, 709-716.

Yuan Li, Liyan Zhang, Linlin Zhao, Ji Feng, Karhoe Loh, Xinqing Zheng and Longshan Lin, 2018. New identification of the moray eel Gymnothorax minor (Temminck & Schlegel, 1846) in China (Anguilliformes, Muraenidae), ZooKeys, 149-161.

Steps involve in Fish diversity estimation

Karan K. Ramteke, Vikas Pathak, Pawan Kumar and Udai Ram Gurjar

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Fish sampling

Before the collection of fish specimen researcher must have information and knowledge of the geographical area of the sampling site, including season of target fish species landing, distribution of fish species, facility to transport, lodging, etc. It is also important to examine the earlier collection of the related fish species to know the different localities from where specimens already have been collected.

If researcher/research scholar understands how fishes are collected and preserved in the field, they can be made better use of the fish specimens and preserved for future. Besides own research, specimens in a museum or in repository serve as valuable representatives of fish species diversity for other research scholar/scientists for decades or even centuries. Therefore, all collections of the fish specimen should be made with the best preservation protocol to secure the highest quality of the preserved fish specimens. Collection of other type fishes allotype, syntype etc.) should be identified against the effort, time and asset invested during sampling.

Permits, regulations, and responsibilities

Taxonomist has to make out with many responsibility and regulations. Under national provisions and regulations on fish species licenses and permits are required for threatened, endangered or protected fish species and it should be considered in the proposed research work.

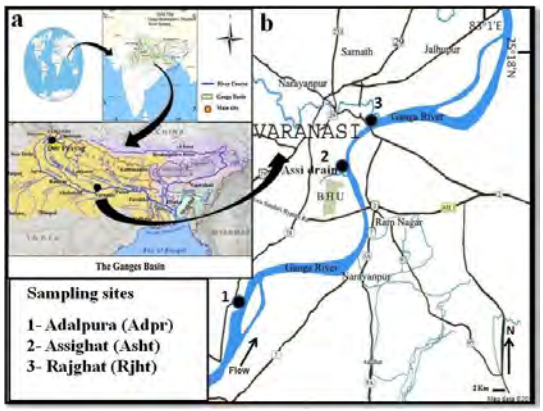
Collecting permits

Prior to the onset of sampling all necessary permits for the proposed fish sampling activities in the research area must be obtained. This includes an institutional/official permit for entry and carry out the research activity, carrying absolute alcohol (for tissue preservation), and fish collection from National Parks or from any prohibited/restricted areas. Illegal fishing (without permission) may lead to fines and other penalties.

Collection of Specimens/data

A specimen without basic and valid data is completely useless for fish taxonomist. Thus, each and every specimen collected must be properly labeled having the following data:

- Geographical Location: Place from where specimens were collected
- Date of Collection: Date on which specimens were collected
- Name of the Collector: Name of the scientist, fisherman
- Coordinates: To be noted using GPS



Specimen numbers

- For fish barcoding, minimum of five specimens of a particular species are considered as the standard number
- For classical fish taxonomy at least thirty specimens of a particular species are considered good.

Preparation: Researchers need during fish sampling for fish taxonomy

- Logbooks
- Dissection kit
- Necessary documents
- Icebox

- Absolute alcohol
- Plastic bags + elastics
- Formalin
- Formalin resistance marker and pencil
- Eppendorf's tubes
- Tags and applicators
- Camera
- GPS

Photography

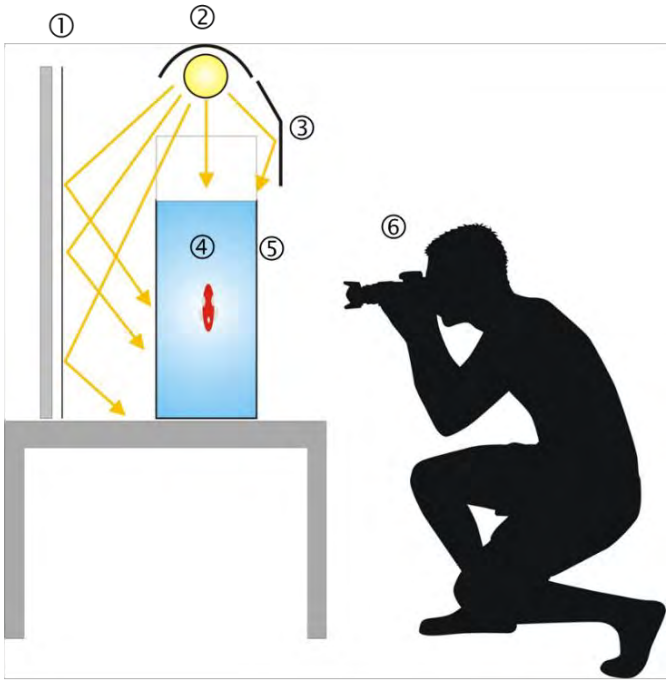
Coloration or pigmentation on the body can be an important character for identification of fish species. Because colors start to disappear or fade rapidly after fish death except black, Therefore, the researcher has only one-time opportunity to record the body color of a fish specimen. That's why photographs should be taken as soon as possible after fish sample collection. The photographs of the left side are highly useful for taxonomic purposes.

All fish specimens of a target species must be photographed and in addition of it, additional specimens (similar in looking, adjacent or type species) may be photographed as well, to cover reproductive status, sexes and other information displaying the diversification within a pecies.

Photos must be digital and color images and at least at a resolution of 1024 X 768 or more and the photographs should be taken of the left side of the fish specimen, except few flatfishes. In the case of the Pleuronectiforms, the eyed side must be shown, with down gill opening. Apart of this, photographs must be taken in lateral aspect when important in other aspects (anglerfishes: dorsal and ventral aspect; flatfishes: right and left side). During photographed fins must be spread as much as possible using pins and alcohol-soaked cotton when needed. The photo should exhibit both Fish and label both.

Tank immersion photography –

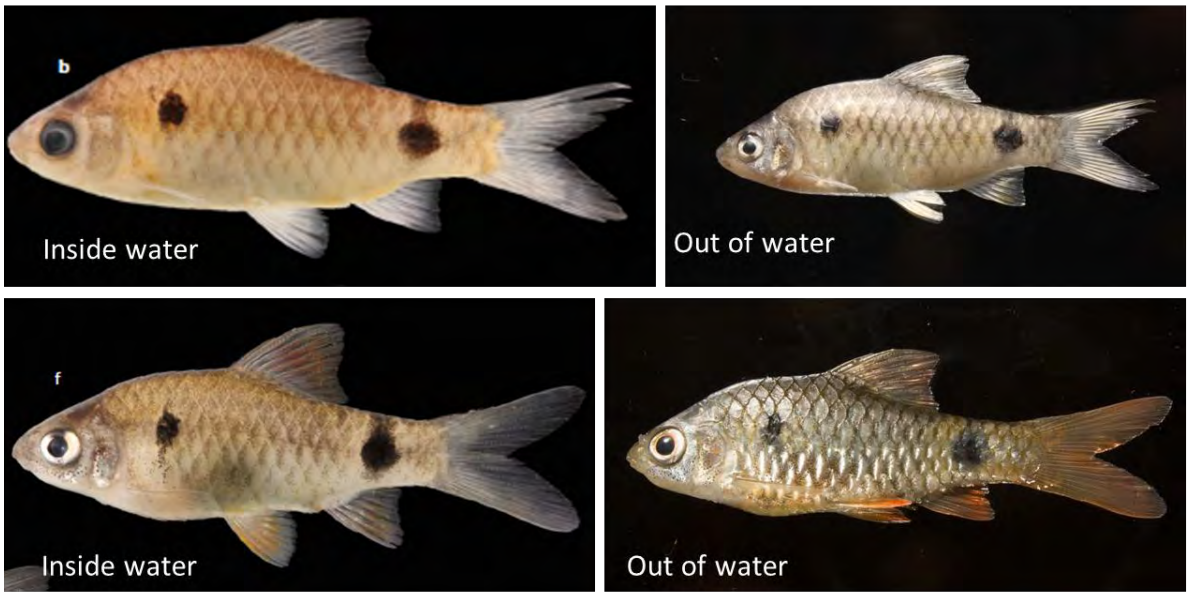
- Photography setup – simple, portable and ready to go
- Underwater photography – aesthetic value but hardly allow to see any details for taxonomy



①Black backdrop/ Silk cloth ②Light source ③Reflector ④Fish ⑤Aquarium ⑥Camera

Field setup -

Inside water vs. Out of water



Required equipment's

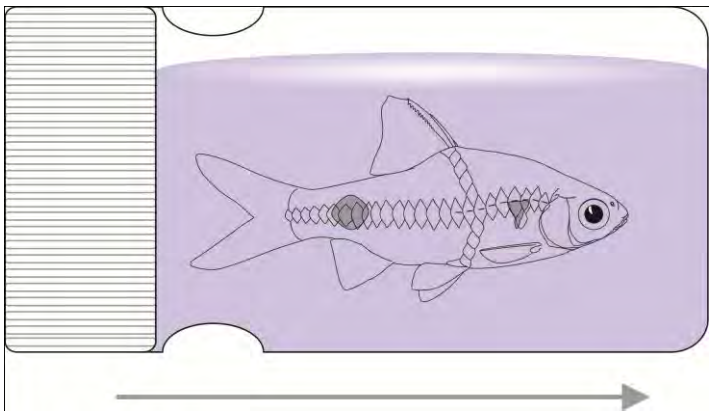
- Camera
- Tripod
- Uniform background
- Pointer (Needles)
- Tweezers
- Tissue paper
- Graph paper
- Notebook

Important points

- Use natural light as a light source (no direct sunlight)
- Pat specimen to avoid reflections
- Take series of the picture with different shutter speed and aperture
- Use unique code
- Consequent: on left side

Fixation and preservation

Huge differences exist between fixation and preservation. Preservation should follow decent fixation. Fixation prevents autolysis by degrading proteins and cross-linking into amino acids by coagulates cell contents and the covalent bonds formation to insoluble substances, while preservation converts the bonding pattern of hydrogen and preserves the fish specimen by dehydrating it. That's why DNA-extraction from ethanol preserved tissues sample is possible, while extraction of DNA from formalin-fixed tissues sample generally yields negligible or only short and degraded gene.



The fixative must penetrate the fish sample as fast as possible to stop decomposition, particularly the guts and guts contents. Pre-fixation must be done within the first 10 minutes of the fish dying. For fixation, the fish should be fully soaked in 10% formalin for large fishes; and 5% for larval and small fishes is recommended. Most of the researchers are even injecting formalin into a body by using a syringe.

After the fixation of fish samples, formalin is replaced by alcohol for storage of specimens for a long duration of time. There are many reasons to convert formalin with alcohol as a preservative medium, a few reasons are followed here 1) formalin forms formic acid on oxidation, which is responsible for demineralization of hard tissues such as a fish skeleton or bone and 2) formalin causes irritation and it is highly poisonous.

Many institutes and research organizations preserve large fish specimens in formalin, which requires more amount of alcohol. When the fish specimens are to be retained in formalin addition of hexamethylenetetramine or sodium hydrogen carbonate (baking soda) in the formalin help to nullify formation of formic acid, as well as use it to fend off the degradation of skeletal tissue.

Things required for preservation:

- Formalin
- Disposable gloves
- Injection needle
- Plastic glasses for protecting eyes
- A plastic tray with tight lid and bottles, 1000 ml, 2000 ml, and 4000 ml.

Reference:

Carpenter, R.G., and Siegler, H.R., 1947, A sportsman's guide to the fresh-water fishes of New Hampshire: Concord, N.H., New Hampshire Fish & Game Department, 87 p.

Scarola, J.F., 1973, Freshwater fishes of New Hampshire: Concord, N.H., New Hampshire Fish and Game Department, 131 p.

Loyacano, H.A., 1975, A list of freshwater fishes of South Carolina: South Carolina Agricultural Experiment Station Bulletin no. 580.

Rohde, F.C., Arndt, R.G., Lindquist, D.G., and Parnel, J.F., 1994, Freshwater fishes of the Carolinas, Virginia, Maryland, and Delaware: Chapel Hill, N.C., The University of North Carolina Press, 222 p.

Chilton, E.W., 1998, Freshwater fishes of Texas: Texas Parks and Wildlife, 98 p.

Hubbs, C., 1972, A checklist of Texas freshwater fishes: Texas Parks and Wildlife Department Technical Service, no. 11, p. 1–11.

Knapp, F.T., 1953, Fishes found in the waters of Texas: Brunswick, Ga., Ragland Studio and Lithograph Printing Company, 166 p.

Morphometric & Meristics Taxonomic Characters

Vikas Pathak, Shashi Bhushan, Karan K. Ramteke and Suman Nama

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

The first step in successfully working with fishes is correct identification. Similar species require in depth examination to discern the few differentiating characteristics. Many times these examinations require accurate measurements and counts of fin ray elements.

Quantitative Characters

Quantitative characters are usually expressed as numbers, these include a measurable and countable characters.

Morphometrics:

Morphometrics are measurable characters or length-based measures of specific body parts, such as total length of the body or diameter of the eye. These characters are usually measured in the millimeter scale.

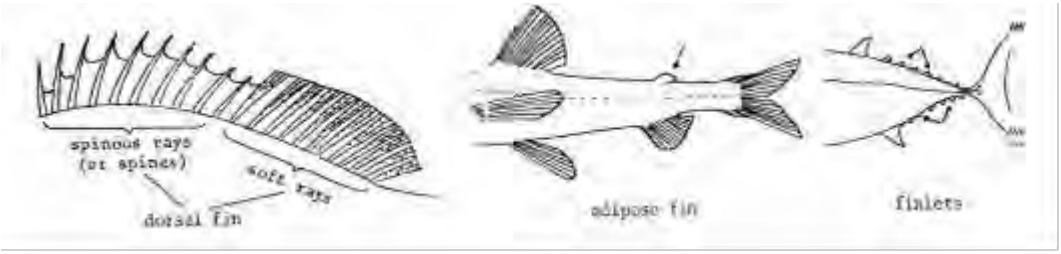
Meristics:

Metistics are counts of things which occur more than once, but a variable number of times between species (and sometimes within species). These include counts of fin elements, i.e.: the number of dorsal fin spines and rays.

NOTE: Spines are hard, pinlike projections, while rays are soft and brush-like occasionally, fins will be a mix of both spines and rays; however, spines are **always** the most forward structures (closest to the head) on the fin.

Differentiate between a spine and a ray

Spines	Rays
Hard and pointed Unsegmented Unbranched Solid	Segmented Sometimes branched Bilateral with left and right halves



Some important considerations:

- Take care to not let the specimens dry out. Use the provided trays with a thin layer of water.
- Frozen fish don't bend easily and their fins often get stuck down. If frozen sample is used, the fish must be completely (or almost) thawed.
- All measurements and counts on paired fins and other structures should be made on the left side of the fish.
- Check and recheck your counts and measurements.
- When making counts it is important to examine the base of each fin where each element inserts into the body. Since most fin elements are difficult to discern without magnification, for this exercise you will use a dissection microscope. Transmitted light usually works best. Since rays are often branched, examining fins at the element tips would result in 2 or more counts for what is only a single element.
- As with most things in ichthyology (fish science) meristic counts are written in a strict format. For example, **Spines** are written using **roman** numerals and **soft-rays** are written using **Arabic** numbers, so:

  D: VII, 10 - is a fish that has *seven* dorsal spines followed by 10 soft rays P_2: 8 - is a fish which has 8 pelvic rays and no spines

It is common to list a range on meristic counts (as D 10-13, A: 10-14, P: 12-15). This illustrates the fin element variability inherent in a single species of fish.

Note: these abbreviation: Dorsal - D, Pectoral – P1, Anal - A, Pelvic – P2, Caudal – C

Identifying External Characters

It presented in the diagram below as a guide to identify all of the fins of fish

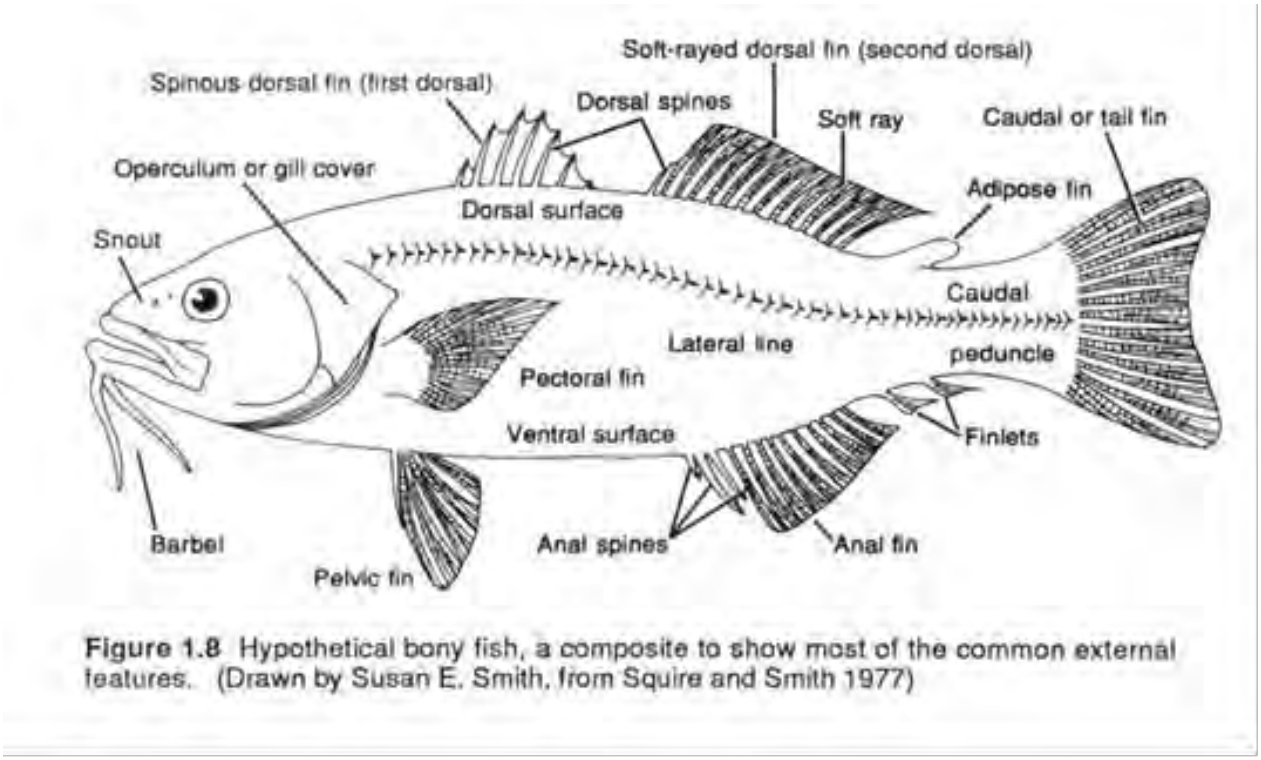
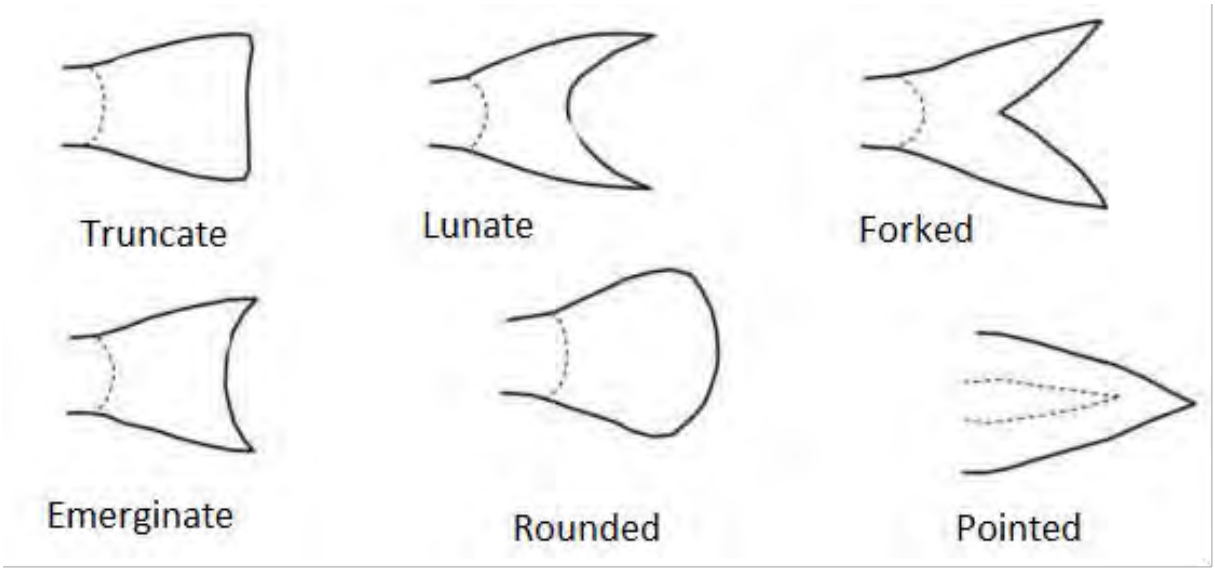
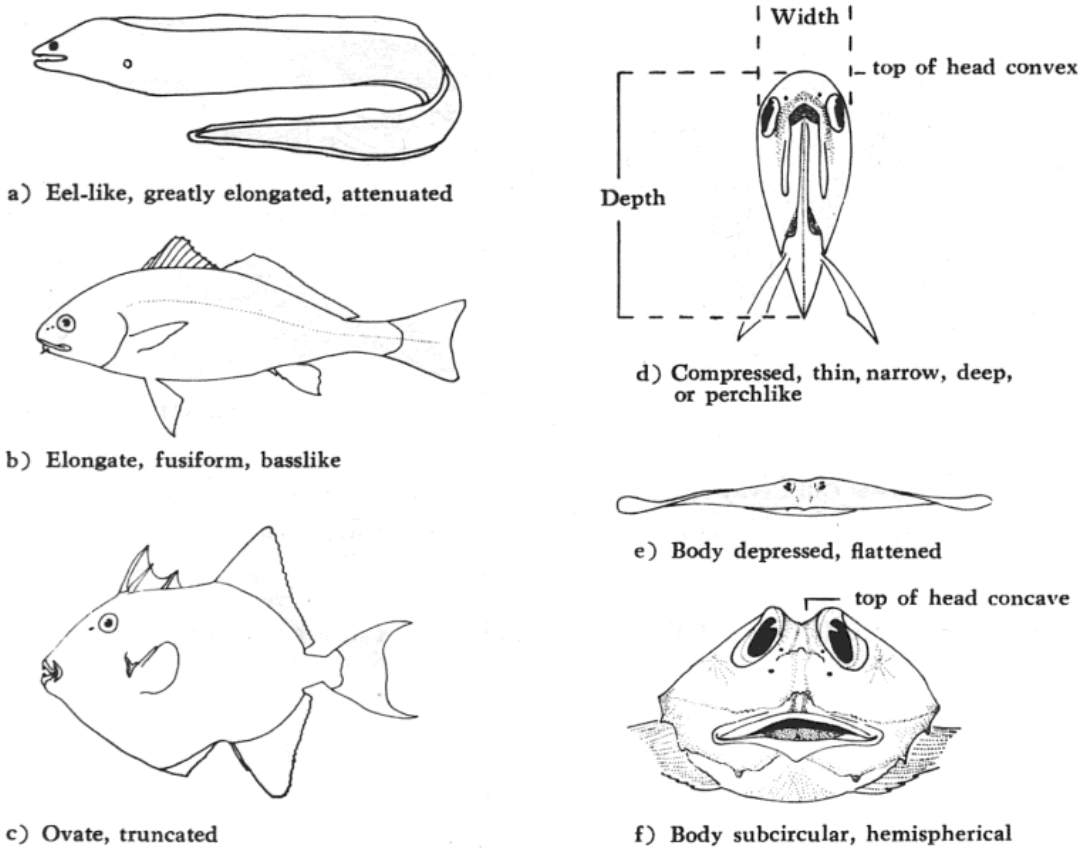


Figure 1.8 Hypothetical bony fish, a composite to show most of the common external features. (Drawn by Susan E. Smith, from Squire and Smith 1977)





a) Eel-like, greatly elongated, attenuated

b) Elongate, fusiform, basslike

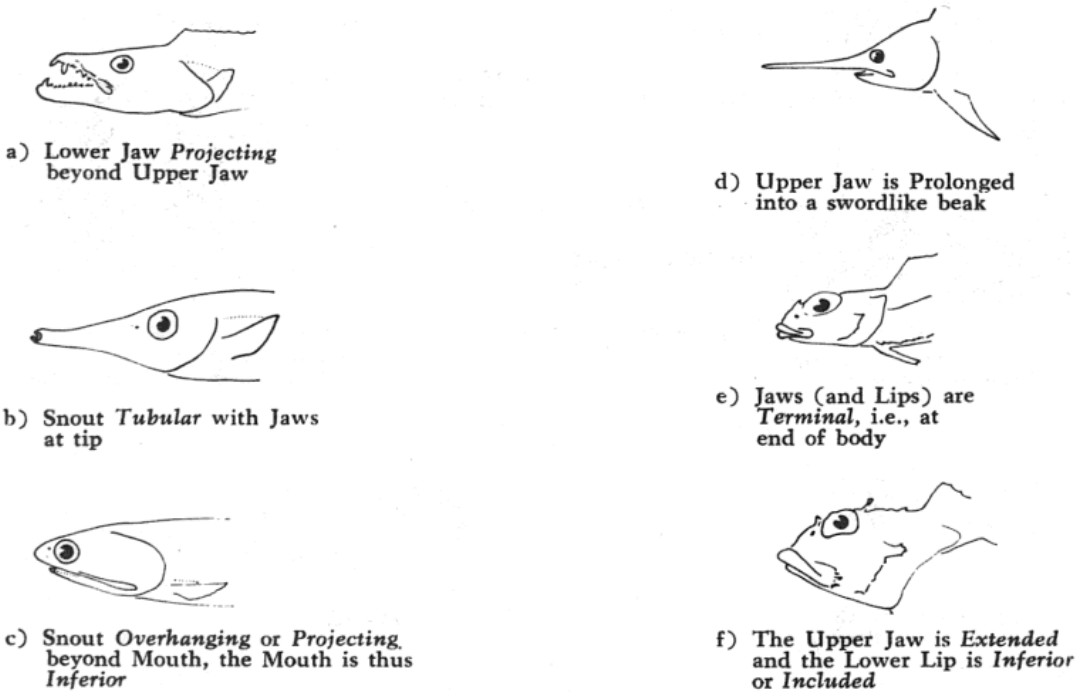
c) Ovate, truncated

d) Compressed, thin, narrow, deep, or perchlike

e) Body depressed, flattened

f) Body subcircular, hemispherical

Some body forms of fishes.



a) Lower Jaw *Projecting* beyond Upper Jaw

b) Snout *Tubular* with Jaws at tip

c) Snout *Overhanging* or *Projecting* beyond Mouth, the Mouth is thus *Inferior*

d) Upper Jaw is Prolonged into a swordlike beak

e) Jaws (and Lips) are *Terminal*, i.e., at end of body

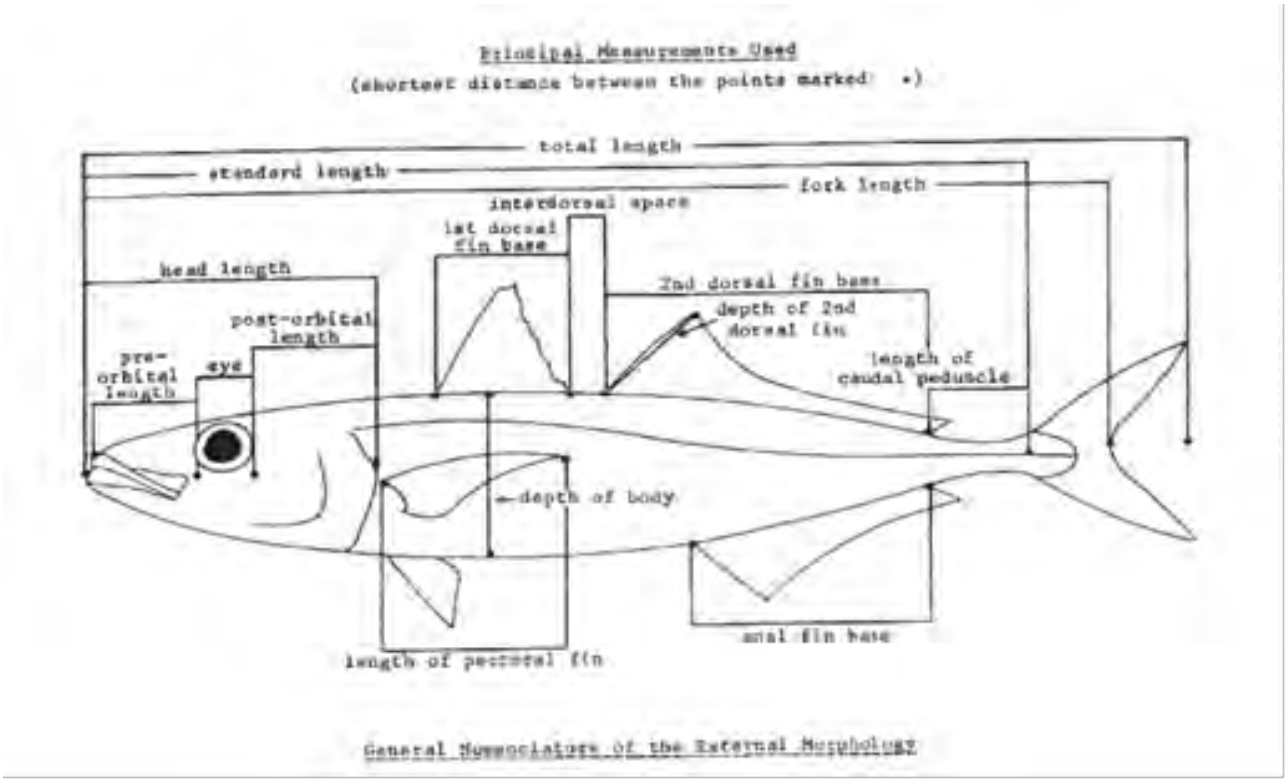
f) The Upper Jaw is *Extended* and the Lower Lip is *Inferior* or *Included*

Terminology of mouth and snout forms.

Morphometric measurements

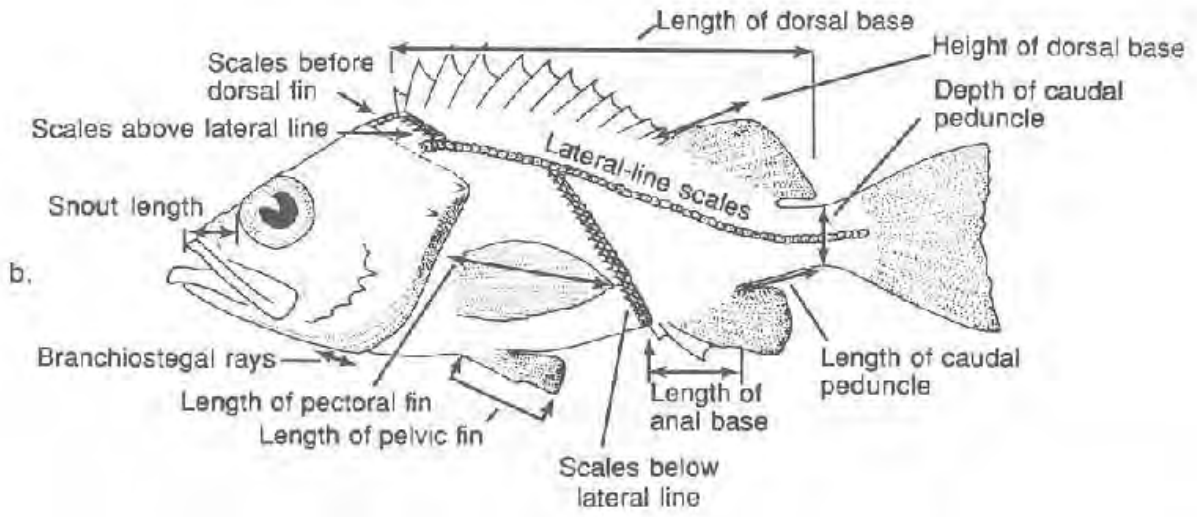
Make all morphometric measurements **before** meristic measurements because the latter will tend to tear up the fins, making them hard to measure. All morphometric measurements can be made with **calipers**. For large fish, you may need a **ruler**

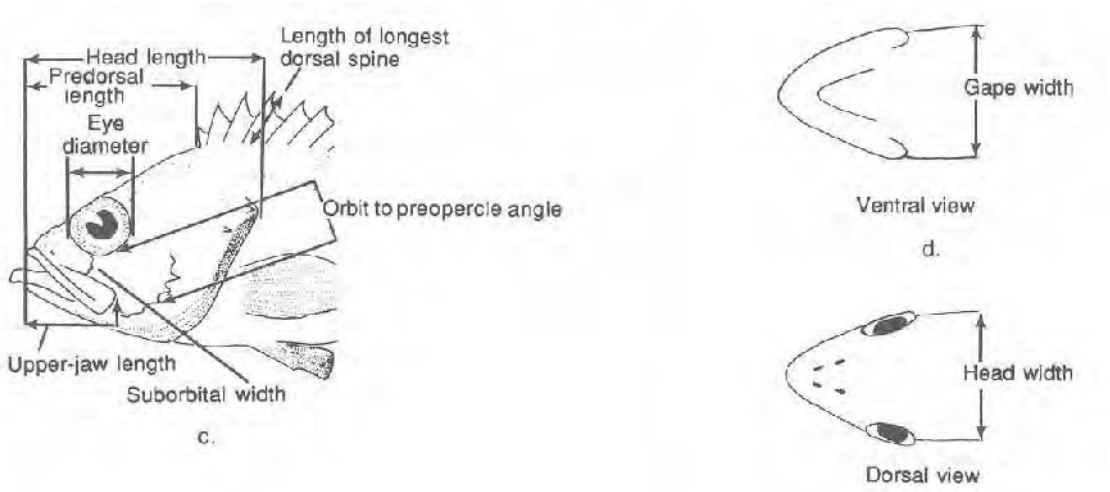
Morphometrics—Given in diagram.



Measurement Type	Length (mm)
Total Length	Tip of mouth to tip of tail fin
Standard Length	Tip of mouth to beginning of tail fin
Fork length	Tip of mouth to the tip of the median caudal fin rays.
Body Depth	The distance from the highest part of the dorsal surface to the ventral surface in a straight, vertical line.

Body Width	Widest measurement side-to-side
Head Length	Tip of mouth to back edge of gill cover
Eye Diameter	The distance between the margins of the eye.
Pre-orbital Length	Snout Length – tip of upper jaw to eye anterior
Post-orbital	The region behind the eye to the rear of the operculum.
Pectoral Fin Length	Upper insertion point of fin to end of longest ray
Tail Height	Top tip to bottom tip of stretched out tail
Depth of caudal peduncle	(refer to diagram)
Length of caudal peduncle	(refer to diagram)
Head width	(refer to diagram)
Gape width	(refer to diagram)





Morphometric Ratios

Although they can be compared to each other directly (for instance, the length of one fish versus another), this does not tell us much because fish change size as they age. However, when compared as ratios (for instance, head length/total length), morphometric measurements can tell us a lot. For instance, cruiser and accelerators might have small head-to-body ratios, whereas maneuverers and especially benthic fish might have large ratios.

Measurement Type				
Body Width/ Standard Length	Body Depth/ Standard Length	Head Length/ Total Length	Predorsal Length/ Total Length	Eye Diameter/ Head Length

Meristics

After you have labeled and measured the fins, you will need to count the spines and rays. Carefully spread the fins, count the spines and rays, and enter these meristic measurements into your lab notebook.

Gill Arch

The gills come in sets of four "arches" on each side of the fish. The red material pointing towards the tail are the gill filaments (used for respiration). The spiny projections pointing towards the mouth are the gill rakers (used for feeding).

The size of gill rakers differs based on the type of prey a species consumes. For example, fish that feed on plankton (Planktivorous) have many very fine gill rakers that are long, whereas fish that feed on large prey have few, small gill rakers

Procedure

- Using your scalpel or your scissors, carefully cut the gill cover back to expose the gills. With your scalpel or your scissors, cut out the **First** gill arch, leaving the others in place.
- Once the gill arch has been removed, place it in a petri dish and GENTLY wash it off. Remove the sticky mucus material which may be coating both the gill filaments and gill rakers.
- Once the arch has been washed, drain the petri dish and place it under the dissecting scope for a better view.
- Now you can count and measure the upper and lower gill rakers.

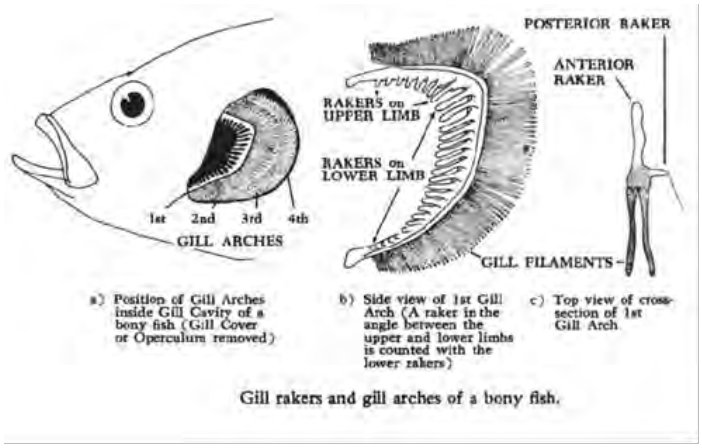


Figure Arrangement and structure of gill rakers and gill arches of a bony fish. (a) location of the gill arches. (b) side view of the first gill arch showing the rakers and filaments. (c) cross section of the first gill arch.

Parts of ctenoid scales:

Some species like *Grammoplites scaber*, *G. suppositus* bear a robust spine on lateral line scales. These body scales of family Platycephalidae play important role in their identification and differentiation.

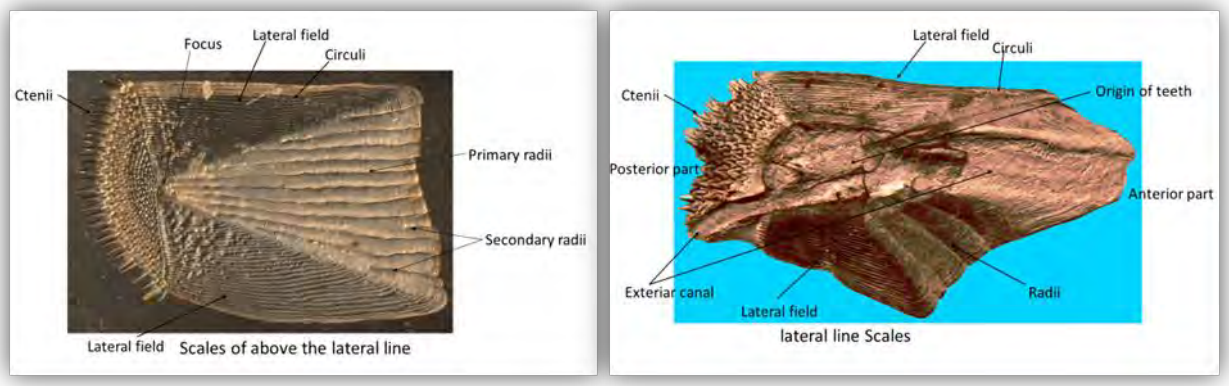


Fig. Parts of body scales

Refrerence:

Casteel R.W., 1972. A key, based on scales, to the families of native California freshwater fishes. Proceedings of the California Academy of Sciences, 39:75-86.

Claytor, R. R. and Maccrimmon, H. R., 1987. Partitioning size from morphometric data: a comparison of five statistical procedures used in fisheries stock identification research. *Can. Techn. Rep. Fish. Aq. Serv.,* 1531:1-23.

Coburn, M. M. and Gaglione, J. I., 1992. A comparative study of percid scales (Teleostei: Perciformes). *Copeia,* 1992(4):986–1001.

Cuvier, G. and Valenciennes, A., 1828-49. *Histoire naturelle des poisons (Vols.22)*.

Day F., 1878. *The Fishes of India* Being a Natural History of the Fishes known to Inhabit the Seas and Freshwaters of India, Burma and Ceylon.. Reprinted 1958, Willialn Dawson and Sons Ltd., London

Elliott, N.G., Haskard, K. and Koslow J.A., 1995. Morphometric analysis of orange roughy (*Haplostethus atlanticus*) off the continental slope of southern Australia. *J. Fish Biol.,* 46: 202-220.

Esmaeili, H. R., Teimory, A. and Hojat-Ansari, T., 2007. Scale structure of cyprinid fish *Capoetada mascina* (Valenciennes in Cuvier and Valenciennes, 1842) using scanning electron microscope (SEM). *Iranian Journal of Science and Technology*, 31(A3): 255 262.

Hisashi Imamura, 2006. Rediagnosis of the Marbled flathead, *Platycephalus marmoraius* (Actinopterygii: Teleostei: Platycephalidae), with comments on the composition of one Type Series. *Species Diversity*, ll: 295-30.

Iwata H., S Niikura, S. Matsuura, Y. Takano and Ukai Y., 1998. Evaluation of variation of root shape of Japanese radish (Raphanus sativus L.) based on image analysis using elliptic fourier descriptors. *Euphytica,* 102: 143-149.

Johnson, G. D. 1984. Percoidei: development and relationships- Ontogeny and systematics of fishes. American *Soc. Ich*. Herp. Special Publication (1): 464–498.

Jones, S. and Kumaran, M., 1980. *Fishes of the Laccadive Archipelago,* Cochin, Mathrubhumi press. 766p.

Knapp L W., 1999. Platycephalidae. In Carpenter K.E. and Niem V. H. eds. FAO identification guide for Fishery purposes.

Knapp L W and Imamura H., 2004. *Sunagocia sainsburyi*, A New Flathead Fish (Scorpaeniformes :Platycephalidae) From Northwestern Australia, Proceedings of *The Biological Society of Washington,* 117:545-550.

Lagler, K. F., 1947. Scale characters of the families of Great Lakes fishes. *Trans. Am. Microscop. Soc.,* 66:149–171.

Laurie C.C., J.R. True, J. Liu, J.M. Mercer, 1997. An introgression analysis of quantitative trait loci that contribute to a morphological difference between Drosophila simulans and D. mauritiana. *Genetics,*145: 339- 348.

Nelson, J.S., 2006. *Fishes of the World.* John Wiley and Sons, New York. 3rd edition. 600pp.

Packer., Laurence., Gibbs, J., Sheffield, C., and Hanner, R., 2009. DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources*, 9: 42- 50.

Steven E. Campana, 2005: Otolith science entering the 21st centuary. *Marine and Freshwater Research*, 56: 485-495.

Tyler S. Zemlak, and Robert Ward, 2009. Barcoding vertebratesDNA barcoding reveals overlooked marine fishes, *Molecular Ecology Resources* 9 (Suppl. 1): 237–242.

Weber, M., and Beaufort, I. F., 1931. *The fishes of Indo-Australian Archipelago*. E.J. Brill Ltd., Leiden. 3: 455pp.

Truss network system

Karan K. Ramteke, Shashi Bhushan Vikas Pathak and Udai Ram Gurjar

ICAR- Central Institute of Fisheries Education Yari-Road Campus, Mumbai

Systematists are often interested in quantifying differences in form among different species, conspecific populations, or ontogenetic stages. Customarily, morphometric data are taken without regard for allometry or its variations among populations or growth stages. It has been common to study growth, for example, by analysis of body length or height or weight only; to describe the shapes of bones by measures of their lengths and widths; to characterize entire forms by relative lengths and breadths of head, trunk, tail, and appendages. Although such measures are deeply entrenched in the methodology of systematics, their usefulness in solving real biological problems may be limited.

Morphometrics is the empirical fusion of geometry with biology. Its methods must explicitly take cognizance of two wholly distinct sources of information-geometric location and biologic homology. The objective of morphometric is to study the associations, causes and effects between the phenotypic traits.

Morphometric analysis consists of the study of landmarks that is the study analyze the data derived from discrete morphometric points, linear distances between points, and geometric relations between points. The second method is the outline method and deal with perimeter shapes.

The routine morphometric traits have limitation in their applications for stock identification due to the large variation is associated with the age. However the landmarks study by employing truss method is useful to overcome the age dependency and help in unbiased stock identification.

Truss network systems constructed with the help of landmark points are powerful tools for stock identification of fish species. A sufficient degree of isolation may result in notable morphological, meristic, and shape differentiation among stocks of a species which may be recognizable as a basis for identifying the stocks. The characteristics may be more applicable for studying short-term, environmentally induced disparities, and the findings can be effectively used for improved fisheries management.

Truss measurements are a powerful tool for the analysis of shape, and generally are designed to cover all, or most, of the animal‘s body. Truss network is more useful and an effective strategy for the descriptions of shape; it has better data collection and diversified analytical tools in comparison to traditional morphometrics method. Thus it is able to

discriminate phenotypic stock because the configuration of the constructed landmarks covers the entire fish body with no loss of information, and it is more sensitive to change

According to Bookstein (2003) there are four principles involved in the land mark study and they are:

1. Land mark locations: In many biological investigations the most effective way to analyze the form of whole biological organs or organism is by recording geometric locations of landmark points. These are the loci that have names viz., origin of the caudal fin etc., as well as Cartesian coordinates. The names are intended to imply true homology (biological correspondence) from form to form. That is landmark points not only have their own locations but also have the same locations in the every other form of the study and in the average of all the forms of a data set.
2. Shape coordinates: Measurements of the shapes of configurations of landmark locations reduces to multiple vectors of shape coordinates. These come in pairs that represent the shape of one triangle of land marks in a manner completely independent of size. That is, the study of covariances of landmark configurations begins when the configurations are represented by multiple triangles.
3. The form of questions: All the main styles of biometric investigation can be realized upon by land mark data by submitting the shape coordinates to multivariate statistical analysis. Although the analysis may be nearly standard in form, the questions to be asked need not be standard at all. They may refer to individual diagnoses, individual forecasts of future form, planning of individual diagnoses, detection or description of group differences, effects of growth or age or size difference on form, detections of patterns of systematic variability of form at diverse geometric scales, or any of a number of possibilities.
4. The form of answers: Results of Morphometric analysis are best represented not in conventional statistical tabulations but instead via geometric diagrams superimposed over a picture or drawing of a typical form.

Methodology

Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected polygons or cells across the body form. Landmarks are points indentified on the basis of local morphological features and chosen to divide the body into functional units.

Truss Using Images

The key set of programs required is Jim Rohlf's tps suite (http://life.bio.sunysb.edu/morph/).

1. tpsUtil- This program will allow you to build tps files.

2. tpsDig- This program will allow you to place landmarks on your images and record scale factors, saving this data in the tps file.

3. PAST (http://folk.uio.no/ohammer/past/)

You will also want a text editor (e.g., Notepad or Word), a spreadsheet editor (e.g., Excel), and a stats package (e.g., SPSS, SAS, many others, even Excel). An image processing program like Photoshop is very helpful.

1. **Preparing the specimen**

a. Place a graph paper on the thermocol. Write the sample ID on the graph paper.

b. Remove traces of water from the specimen using cloth/blotting paper.

c. Place the specimen on the graph and pin the fins erected so that it makes the origin and insertion points visible.

2. **Imaging**

a. Leave space around your image during photography. If your image fills the frame, it will likely be distort¬ed near the edges. You can still crop your image afterwards (e.g., in Photoshop).

b. Take photos at max available resolution. Storage is cheap, so don't needlessly shrink your images just to save disk space. You may want that information later. Images should be jpegs or uncompressed tiff files. Other formats are okay, but you risk having problems with the analysis programs.

c. Always use a scale bar. Take your photos with a ruler visible in the picture, preferably not on the edge of the image (to avoid distortion).

3. **Building a tps file**

a. Place all of the images that you want to use (or copies of them) in the same folder.

Possible issues: tps programs have had issues with really long file paths (the file name plus all of the names of the folders it's in). To avoid this issue, place your images in My Documents or in the C:\ directory in a folder with a short name (e.g., –analysis"). You can move them out after you're done.

b. Open tpsUtil (Start > All Programs > tps > tpsUtil)

c. Click on –Select an operation" and choose –Build tps file" from the drop-down list.

d. Select your input directory:

i. Click –Input" (which should now be visible).

ii. Find your directory of images.

iii. Double-click one image in that directory (instead of –Data file = ?", it should show the path to your images)

e. Name your output file

i. Click –Output"

ii. Enter a name that ends in –.tps". Save this file in the folder with your images (instead of –Output file = ?", it should show the path to your images)

f. Build the tps file

i. Click –Setup" (which should now be visible).

ii. Checked images will be used to build your tps file. Check/uncheck as needed. If the folder contains only the relevant images, your list should be fine.

Note: if you click –include path", you can move the tps file anywhere and it will still –find" the images, but then you can't move the images. If you leave it unselected, you have to keep the tps file in the folder with the images, but you can move that folder anywhere you want.

iii. Confirm that you have a file named –[something].tps" under –File to be created". If not, go back to step „e'.

iv. Click –Create"

v. Click –Close" to exit tpsUtil.

You should now have a file that you can open in tpsDig.

What a raw tps file looks like (open in a text editor like Notepad):

LM=0

IMAGE=MSB_125_mackerel.JPG

LM=0

IMAGE=MSB_126_mackerel.JPG

Your specimens (given by the IMAGE= names) currently have zero landmarks (LM=0).

4. **Landmarking the specimens**

In this step you place landmarks on your images and save them into your tps file.

a. Open tpsDig (Start > All Programs > tps > tpsDig

b. Open your raw tps file (File > Input Source > File...)

You should now be able to scroll through all of your images using either the right and left arrow keys (on your keyboard) or the red arrow buttons on the top left hand corner of the navigation screen. The file name is listed at the bottom of the screen, and information on the number of landmarks will appear there as you begin collecting data. The help files are worth a look.

c. Place landmarks. Select the crosshairs icon. Left-click to place a landmark at the appropriate location.

Remember: all landmarks must be placed in sequence. If you click a landmark (LM) with the arrow tool, the LM number will be shown at the bottom of the screen. If you've screwed up the sequence, you can move the LMs with the arrow tool. Right-clicking on a landmark

with the arrow tool will allow you to delete that LM or insert a new LM before the selected LM.

Make sure you know what your landmarks are before you start and be ready to make adjustments them after you start. This happens sometimes with ambiguous (e.g., non-Type I) landmarks. Consistency in landmark placement is critical, so it is important to spend a good amount of time preparing and thinking about where your landmarks will go.

d. Set scale. Go to Options > Set Scale. In the pop-up window select the length of your scale (e.g., if you have a 10cm ruler, enter 10.0). In your image, place the cursor (now an arrow with a cross) on one end of your ruler. Left-click, then set the arrow at the other end and left-click again. The scale window should now show your scale factor. Click OK.

Note: The ability to set scales in tpsDig is extremely useful and it should always be used. It allows you to photograph specimens without worrying about the distance between the lens and the specimen by using the scale bar in your images and inserting a scale adjustment into the tps file. Even if you know that your lens was always at an equal distance, it does not hurt to set a scale. You can set the scale for each specimen as you landmark, or come back to it later.

e. Save your landmark data. File > Save data > Save > Overwrite. Because these programs are made by indi¬viduals and not corporations, they are prone to minor glitches (that can make you lose valuable informa¬tion). Therefore, save often.

Repeat this process for each specimen. Your tps file should now look like this:

LM=4
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
IMAGE=MSB_125_mackerel.JPG
ID=1
SCALE=0.006664

Note that each specimen starts with –LM=" and ends with the scale factor. Occasionally, you may find yourself manually editing files in Notepad. It can be confusing having the specimen name at the end of the specimen data. Be careful about this. The ID number doesn't matter; you can concatenate files so that there are multiple specimens with the same ID.

5. Extracting truss measurements

a. Open the tps file in past software.

b. The data comes in XY coordinates.

c. Select the data, go to geomet and click on distances from all landmarks.

d. Enter 2 for the dimension.

e. The XY coordinates gets converted to distances between the landmarks.

f. Select the necessary distances and delete the rest.

6. Data analysis

The analysis can be done in any of the statistical packages available.

Methodology for the truss morphometry

Karan K. Ramteke, Vikas Pathak, Abhijit Mallik and Suman Nama
ICAR- Central Institute of Fisheries Education, Yari-Road Campus, Mumbai

❖ Sampling

- Sampling sites should be selected from the distributional range of the species under study. It should be taken care that the sites are at least 200km apart.
- Sampling should be carried out preferably during spawning seasons, when mixing among putative stocks is minimal.
- Sampling from the selected sites should be done within 2 months so as to avoid seasonal variation within/between samples.
- The landmarks and the truss distances should be decided prior to sampling in order to decide the sample size. The sample size should be three times the number of variables (truss distances). For e.g., if the truss network contains 30 truss distances, the sample size to be taken is 90.
- Record the sex, maturity stage and weight of gonad of the specimens as ripe gonads can contribute up to 40% of female body mass.
- Location information such as depth, salinity, temperature, and sea floor substrate may also be recorded. Such data serve as useful covariates and possibly causal factors for exploring patterns of morphometric variation.
- Data on gear type and mesh size will also be useful.
- The specimens have to be frozen at -20°C for 72 hours since the shrinkage will be much less after 72 hours.
- The specimens should be whole, without body shape deformation or broken parts.
- Discard the specimens that are damaged.

❖ Choice of Characters

- Landmarks should be anatomical points, representing the same developmental feature among specimens, and should be easily located.
- The most effective landmarks are those defined by the intersection of different tissues, such as insertion points of fins and anal pores.
- The network should resemble the shape of the specimen from which it is derived.

❖ Positioning

- Place a drawing sheet between two thin thermocol sheets.
- Remove trace of water from fish using blotting paper.

- Position the fish on the thermocol and keep the body posture and fins into a natural position.
- Measurements are taken on one side of the fish throughout sampling.
- Label each drawing sheet to identify the particular specimen, specifying the date, location, sex, length, weight, and sample number.

❖ **Pinning**

Landmarks selected for the truss network should be homologous, representing the same developmental feature among specimens, and should be easy to locate (Winans, 1987; Bookstein, 1990). The most effective way for defining landmarks is by the location of intersection of different tissues, such as insertion points of fins and anal pores.

- Select landmarks around the outline of the fish form.
- Mark each landmark by piercing the drawing sheet with long round head pins.
- Additional data, such as eye diameter and head width, can also be recorded and added in the truss data.

❖ **Networking**

- Remove the drawing sheets
- Interconnect the landmarks by drawing straight lines with help of a pencil, to form a series of connected polygons called _truss network'.
- Measure the truss distances with the help of a digital vernier caliper.
- The measurements should be done up to 2 decimal places.

Or

Digitizing

Digital records of specimens provide a complete archive of body shape and give the opportunity for repeated measures if required (Cadrin and Friedland, 1999).

The acetate sheet is placed on a light box, and a camera, connected to a monitor, video and computer, is set at the top of the light box, and the image is stored on the screen of the monitor to view interlandmark distances. The X-Y coordinate value for the positions of landmarks is digitized for each fish by Measurement TV Program (Data Crunch Product). Alternatively, an X-Y coordinate digitizing pad can also be used to establish a reference set of X and Y axes to view interlandmark distances. All measurements are transferred to a spreadsheet file (e.g. Excel or Lotus), and X-Y coordinate data is transformed into linear distances by computer (using the Pythagorean theorem) for subsequent analysis. **(according to turan's paper)**

Or

<u>Truss Using Images</u>

The key set of programs required is Jim Rohlf's tps suite (http://life.bio.sunysb.edu/morph/).

1. ***tpsUtil***- This program will allow you to build tps files.
2. ***tpsDig***- This program will allow you to place landmarks on your images and record scale factors, saving this data in the tps file.
3. PAST (http://folk.uio.no/ohammer/past/)

You will also want a text editor (e.g., Notepad or Word), a spreadsheet editor (e.g., Excel), and a stats package (e.g., SPSS, SAS, many others, even Excel). An image processing program like Photoshop is very helpful.

1. Preparing the specimen

a. Place a graph paper on the thermocol. Write the sample ID on the graph paper.

b. Remove traces of water from the specimen using cloth/blotting paper.

c. Place the specimen on the graph and pin the fins erected so that it makes the origin and insertion points visible.

2. Imaging

a. Leave space around your image during photography. If your image fills the frame, it will likely be distorted near the edges. You can still crop your image afterwards (e.g., in Photoshop).

b. Take photos at max available resolution. Storage is cheap, so don't needlessly shrink your images just to save disk space. You may want that information later. Images should be jpegs or uncompressed tiff files. Other formats are okay, but you risk having problems with the analysis programs.

c. Always use a scale bar. Take your photos with a ruler visible in the picture, preferably not on the edge of the image (to avoid distortion).

3. Building a tps file

a. Place all of the images that you want to use (or copies of them) in the same folder.

Possible issues: tps programs have had issues with really long file paths (the file name plus all of the names of the folders it's in). To avoid this issue, place your images in My Documents or in the C:\ directory in a folder with a short name (e.g., –analysis"). You can move them out after you're done.

b. Open tpsUtil (Start > All Programs > tps > tpsUtil)

c. Click on –Select an operation" and choose –Build tps file" from the drop-down list.

d. Select your input directory:

i. Click –Input" (which should now be visible).
ii. Find your directory of images.
iii. Double-click one image in that directory (instead of –Data file = ?", it should show the path to your images)

e. Name your output file

i. Click –Output"
ii. Enter a name that ends in –.tps". Save this file in the folder with your images (instead of –Output file = ?", it should show the path to your images)

f. Build the tps file

i. Click –Setup" (which should now be visible).
ii. Checked images will be used to build your tps file. Check/uncheck as needed. If the folder contains only the relevant images, your list should be fine.
Note: if you click –include path", you can move the tps file anywhere and it will still –find" the images, but then you can't move the images. If you leave it unselected, you have to keep the tps file in the folder with the images, but you can move that folder anywhere you want.
iii. Confirm that you have a file named –[something].tps" under –File to be created". If not, go back to step _e'.
iv. Click –Create"
v. Click –Close" to exit tpsUtil.

You should now have a file that you can open in tpsDig.

What a raw tps file looks like (open in a text editor like Notepad):

LM=0
IMAGE=MSB_125_mackerel.JPG
LM=0
IMAGE=MSB_126_mackerel.JPG

Your specimens (given by the IMAGE= names) currently have zero landmarks (LM=0).

4. Landmarking the specimens

In this step you place landmarks on your images and save them into your tps file.

a. Open tpsDig (Start > All Programs > tps > tpsDig

b. Open your raw tps file (File > Input Source > File...)
You should now be able to scroll through all of your images using either the right and left arrow keys (on your keyboard) or the red arrow buttons on the top left hand corner of the navigation screen. The file name is listed at the bottom of the screen, and

information on the number of landmarks will appear there as you begin collecting data. The help files are worth a look.

c. Place landmarks. Select the crosshairs icon. Left-click to place a landmark at the appropriate location.

Remember: all landmarks must be placed in sequence. If you click a landmark (LM) with the arrow tool, the LM number will be shown at the bottom of the screen. If you've screwed up the sequence, you can move the LMs with the arrow tool. Right-clicking on a landmark with the arrow tool will allow you to delete that LM or insert a new LM before the selected LM.

Make sure you know what your landmarks are before you start and be ready to make adjustments them after you start. This happens sometimes with ambiguous (e.g., non-Type I) landmarks. Consistency in landmark placement is critical, so it is important to spend a good amount of time preparing and thinking about where your landmarks will go.

d. Set scale. Go to Options > Set Scale. In the pop-up window select the length of your scale (e.g., if you have a 10cm ruler, enter 10.0). In your image, place the cursor (now an arrow with a cross) on one end of your ruler. Left-click, then set the arrow at the other end and left-click again. The scale window should now show your scale factor. Click OK.

Note: The ability to set scales in tpsDig is extremely useful and it should always be used. It allows you to photograph specimens without worrying about the distance between the lens and the specimen by using the scale bar in your images and inserting a scale adjustment into the tps file. Even if you know that your lens was always at an equal distance, it does not hurt to set a scale. You can set the scale for each specimen as you landmark, or come back to it later.

e. Save your landmark data. File > Save data > Save > Overwrite. Because these programs are made by individuals and not corporations, they are prone to minor glitches (that can make you lose valuable information). Therefore, save often.

Repeat this process for each specimen. Your tps file should now look like this:

LM=4
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000

IMAGE=MSB_125_mackerel.JPG

ID=1

SCALE=0.006664

Note that each specimen starts with –LM=" and ends with the scale factor. Occasionally, you may find yourself manually editing files in Notepad. It can be confusing having the specimen name at the end of the specimen data. Be careful about this. The ID number doesn't matter; you can concatenate files so that there are multiple specimens with the same ID.

5. Extracting truss measurements

a. Open the tps file in past software.
b. The data comes in XY coordinates.
c. Select the data, go to geomet and click on distances from all landmarks.
d. Enter 2 for the dimension.
e. The XY coordinates gets converted to distances between the landmarks.
f. Select the necessary distances and delete the rest.

❖ **Data Entry (for SAS software)**

- Create a data set by entering the data in Microsoft Excel with variables in columns and observations in rows.
- Naming data sets and variables
- Names must be 32 characters or fewer in length.
- Names can contain only letters, numerals, or underscores (_). No special characters like % $! * & # @
- Names can contain upper- and lowercase letters.
- All the samples' data can be combined in one spreadsheet, with location, coast, zone and sex as variables.

Data analysis

When all samples for all populations have been taken from the same ages there is no need to eliminate the size effect in the data set. Otherwise, an important stage in the data preparation for morphometric analyses is to eliminate any size effect in the data set when comparing fish of different sizes. Variation should be attributable to body shape differences, and not related to the relative size of the fish. Therefore, transformation of absolute measurements to size-independent shape variables is the first step of the analyses. Several

transformation methods previously shown to be effective in removing such size-dependent variation can be compared (24). The transformations are:

I) RATIO: Madj=M/SL, ie. division by standard length.

II) LGRATIO: Madj=logM/logSL = the log of ratio

III)ALLOM1: Madj=logM-b1(logSL-logSLmean)

IV)ALLOM2: Madj=logM-b2(logSL-logSLmean)

Where:

M: original truss measurement

Madj: size adjusted truss measurement

SL: standard length of fish

SLmean: overall mean of standard length

b1: coefficient of the overall linear regression of logM against logSL

b2: average pooled within-sample coefficient of the regressions of log M against log SL

Base-10 logarithms are used for all variables.

Standard length is used for all cases, since it correlates strongly with other morphometric characters (24, 25, 26). The efficiency of size adjustment transformations can be assessed by testing the significance of correlations between the transformed variables and the standard length. A significant correlation indicates an incomplete removal of size effects from the data. Standard length (landmark distance between a and f, Figure 1) must be excluded from the analyses. The second way of eliminating the size effect in principal components analysis (PCA) is to eliminate the first principal component (PC) from the analysis, as the first PC expresses size variation, while the others express genuine variation among stocks. Weight unit can also be used for eliminating the size effect (27), though weight is a power of length (28). In this way, each morphometric measurement is divided by the individual gutted weight prior to multivariate analysis.

Morphological differentiation may vary between the sexes in some fish species: Creech (1993) reported greater variation between two sandsmelt species in females than in males. Therefore, the interaction between variables and sexes should also be tested. In the case of any significant correlation, females and males should be treated separately in multivariate analyses to remove the effect of sex from the result.

The transformed data should be standardised to a 0 mean and a standard deviation of 1 and can be submitted to a principal components analysis and a multiple-discriminant function analysis (DFA) or canonical analysis (CA) using a statistical package program (e.g. SPSS, SYSTAT) and graphs can also be generated using these programs. The transformed data are also used for other statistical analyses (e.g. Analysis of Variance (ANOVA), and Multivariate Analysis of Variance (MANOVA).

Multivariate analyses

Multivariate techniques simultaneously consider the variation in several characters and thereby assess the similarities between samples. PCA requires no a priori grouping of individuals but combines and summarizes the variation associated with each of a number of measured variables into a smaller number of principal components (PCs) which are a linear combination of the variables that describe the shape variations in the pooled sample.
Correlations between original variables and the principal components (component loading) can be used to interpret the importance of individual variables in the description of the variation of the data set.

CA is used to discriminate the samples according to the variables. CA requires a priori grouping of samples, calculates a function discriminating between samples of known identity and then reclassifies the individuals into the designated groups on the bases of this function. The percentage of correctly classified individuals gives a measure of the morphological distinctness of the samples.

Principal components and canonical analysis can be used to produce graphs to visualise relationships among the individuals of groups by plotting population centroids of 95% confidence ellipses of first two canonical functions (CFs) and PCs The measurements with high loadingsin CA are between-sample diversity, and hence differ from those in PCA (which have total diversity). Each principal component contains the percentage of total variance of all variables. But in CA, each function contains the percentage of the total between-groups variability. Therefore, CA is used to describe the pattern of phenotypic differentiation among samples.

Univariate analysis of variance can be used to compare the variation among samples for size-adjusted truss measurements. Post hoc multiple comparison tests can also be performed to find the number of significant morphometric characters between pairs of samples. The number of significantly different measurements among groups is an additional indication of the degree of group separation. The effect of sex on the truss measurements should also be tested using univariate statistics. Multivariate analysis of variance can be performed to test the significance of differences among the samples in the data set.

The analysis can be done in any of the statistical packages available.

Data Analysis in SAS

- Open SAS program
- Five main SAS windows open: the Explorer, Results, Program Editor or Editor, Log, and Output windows

- In the Explorer window, you can view and manage your SAS files and create shortcuts to files that are not formatted by SAS.
  - Program editor window can be used to enter, edit, and submit SAS programs.
  - The Log window displays messages about your SAS session and any SAS programs that you submit.
  - The Output window displays the output from SAS programs that you submit. It automatically opens or moves to the front of your display when you create output.
  - The Results window helps you navigate and manage output from SAS programs that you submit. You can view, save, and print individual items of output.
- Import the prepared data set from MS-Excel. [Open File ⟶ Import data]
- Type program in the Editor window or open previously saved program file. [Open File ⟶ Open program]
- Test the data for ***normality distribution by PROC UNIVARIATE***

```
proc univariate data=mackerel plot normal;
var sl;
histogram sl/normal;
run;
```

- Check for outliers in the data with the help of list of highest and lowest values, _stem and leaf' and box plots in the output. Remove the outliers, if any.
- Save the data.
- Sort the data according to location by PROC SORT

```
proc sort data=cas;
by location;
run;
```

- Age/size dependent correction from the truss variables

The general approaches to isolate shape from size variation include

  - Expression of variables as ratio of fish length (Casselman et al., 1981; King, 1985)
  - $M_{adj} = M/SL$, ie. division by standard length
  - logratio: $M_{adj} = logM/logSL$ = the log of ratio
  - division by geometric mean of all variables for a particular specimen (Mosimann, 1970; Jungers et. al., 1995)
  - adjustments by geometric mean regressions (Meng and Stocker, 1984)
  - covariate adjustments based on fish length (Waldman and Fabrizio, 1994)
  - regression of each truss variable against a measure of body size and estimating residual truss variation. The measure of body size can be total length, standard length

or any possible standard length. Reist (1985) suggested the following formula for size correction,

- $M_{trans} = logM - \beta\ (logSL - logSL_{mean})$
- where M_{trans} is the transformed measurement, M the original measurement, β the within-group slope regressions of the logM vs. logSL, SL the standard length of the fish, and SL_{mean} the overall mean of the standard length.
- $M_t = M_o\ (Pe_{ov}/P_o)^b$ where, M_t: standardized measurement, M_o: observed truss distance, Pe_{ov}: the overall mean perimeter for all fish in all groups, P_o: the perimeter of a specimen calculated as the summation of the contiguous distances of landmarks, and b: slope of the regression of log M_o to log P_o for each subset (Agüero and Rodríguez, 2004).
- Growth-invariant discriminant analysis (Burnaby, 1966)
- Multiple-group PC analysis, or MGPCA. Unlike PC analysis, which can be applied only to a single group, MGPCA allows the user to extract within-group size effects when two or more groups are considered (Thorpe, 1988)

- Correlation between the truss variables before and after transformation
  - This is to check whether the size effect on the truss variables were effectively removed or not after the transformation.
  - Before the transformation the variables will be highly correlated to each other
  - After transformation the correlation between the variables should be insignificant.
  - The following proc statement is used

```
proc corr data=mackerel;
var m:;
run;
```

- Principal component analysis of the transformed truss variables
  - Following is the SAS program for PCA analysis

```
options linesize=74 pagesize=54 nodate;
goptions reset=all;
legend1 frame /*cframe=ligr*/ cborder=black
        position=center value=(justify=center);
axis1 minor=none label=(angle=90 rotate=0);
axis2 minor=none;
symbol1 value=star color=green;
symbol2 value=triangle color=red;
```

```
%let inputs= m:;
title'Principal Component Analysis of different stocks of Indian Mackerel';
proc princomp data=mackerel cov n=2 out=scores;
var m:;
run;
proc sort data=scores;
by location;
run;
title 'Truss PCA analysis for different stocks of Indian Mackerel';
proc gplot data=scores;
plot prin2*prin1= location/frame /*cframe=ligr*/
     vaxis=axis1 haxis=axis2 legend=legend1;
run;
```

- In PCA, the number of components extracted is equal to the number of variables being analyzed. Only the first few components will be important enough to be retained for interpretation.
- The output provides the eigenvalue table. An eigenvalue represents the amount of variance that is accounted for by a given component.
- Determining the number of _meaningful' components
  - Scree test (Cattell, 1966): plot the eigenvalues associated with each component and look for a _break' between the components with relatively large eigenvalues and those with small eigenvalues. Those components that appear before the break are assumed to be meaningful and are retained; those appearing after the break are assumed to be unimportant and not retained.
- Interpreting the rotated solution: this involves identifying the variables that demonstrate high loadings (usually more than 0.4) for a given component and determining what these variables have in common. If a given variable has a meaningful loading on more than one component, scratch that variable out and ignore it from the interpretation. Repeat the procedure for all the variables. Again repeat this process to name the remaining retained components.

- Factor analysis of the transformed truss variables (Hatcher, 1994)
  - Factor analysis may be appropriate when you have obtained measures on a number of variables, and want to identify the number and nature of the underlying factors responsible for covariation in the data. SAS proc statement for factor analysis is given below

```
Proc factor data=mackerel
simple
heywood
method=ml
preplot
nfactors=2
out=factorout
plot
rotate=varimax
scree;
Title'Factor Analysis';
var m:;
run;
proc sort data=factorout;
by location;
run;
options linesize=74 pagesize=54 nodate;
goptions reset=all;
legend1 frame /*cframe=ligr*/ cborder=black
position=center value=(justify=center);
axis1 minor=none label=(angle=90 rotate=0);
axis2 minor=none;
symbol1 value=star color=green;
symbol2 value=triangle color=red;
%let inputs= m:;
title'Factor Analysis M=ml, R=varimax Indian Mackerel';
proc gplot data=factorout;
plot factor2*factor1=location/frame /*cframe=ligr*/
legend=legend1 vaxis=axis1 haxis=axis2;
run;
```

- Initial extraction of factors: maximum likelihood method is usually preferred as it provides more accurate parameter estimates, and also provides a significance test to help solve the number of factors problem.
- The number of factors extracted is equal to the number of variables being analyzed.

- Output gives eigenvalue which represents the amount of variance that is accounted for by a given factor.
- Determining the number of ‗meaningful' factors to retain
  - Scree test (Cattell, 1966)
- Rotation to a final solution: varimax
- Interpreting the rotated solution: the rotated factor pattern matrix gives the pattern loadings. Loadings equal to or greater than 0.40 are considered as meaningful loadings (Stevens, 1986). If a given variable has a meaningful loading on more than one factor, scratch that variable out and ignore it from the interpretation. Repeat the procedure for all the variables. Again repeat this process to name the remaining retained factors.

- **Discriminant Function Analysis**

PROC DISCRIM procedure of SAS is used to carry out the classification by the cross-validation of the discriminant analysis of the data. It is used to determine which continuous variables discriminate between two or more naturally occurring groups.

proc discrim data=truss outstat=outtruss method=normal pool=yes list crossvalidate;
class location;
priors prop;
id slno;
var Pt3t9, Pt2t11,Pt3t4, Pt3t9, Pt3t10;
run;

Fatty acid analysis: a chemotaxonomic tool for fish stock identification

Layana. P. and Vignaesh Dhanapalan

ICAR – Central Institute of Fisheries Education, off – Yari road, Versova, Mumbai

Introduction

A stock can be defined as a population or portion of a population of which all members are characterized by similarities which are not heritable, but are induced by the environment, and which include members of several different subpopulations (Maria et al., 2014). Identification and assessment of stock structure is essential in fisheries management. Chemically, fatty acids are sigma-bonded carbon chains with a carboxylic acid functional group at one end. Different species of fish have their characteristic fatty acid profiles in different tissues. In tissues the fatty acids are present as different classes of lipids such as triacylglycerides, phospholipids, wax esters, cholesterol etc. However, the composition of the tissue fatty acids of fish may be influenced by various factors such as diet, age, maturity, spawning, temperature and salinity of water and therefore the variability in fatty acid composition is very large between and within the species. This has remained as one of the limitations when using fatty acid profiles of muscle tissue as natural markers to differentiate species, subspecies or stocks. However, the assumption among the researchers is that the composition of fatty acids in membrane phospholipids is genetically controlled and stable over time as they are relatively robust to dietary changes. Studies have revealed that the lipid composition of cardiac skeletal muscle has a high level of phospholipids in the membrane and its particular fatty acyl structure restricts the ability of the acyl chains to reflect diet (Hishikawa et al., 2008). Hence fatty acid profile of cardiac skeletal muscle can be considered as a highly stable trait and may serve as natural markers for the identification of stocks and to establish taxonomic relationship among different species.

Fatty acid profiling using Gas Chromatography- Mass spectrometry (GC-MS)

GC is one of the most convenient methods for the identification of fatty acids present in sample phospholipids or total lipids. It is a chromatographic technique useful for separating volatile organic compounds such as fatty acid methyl esters (FAME). Here, organic compounds are separated due to the differences in their partition behavior (affinity) between

the mobile gas phase and the stationary phase in the column. The stock identification is possible based on different parameters such as number of fatty acids present, presence and absence of a particular fatty acid, the quantity of each fatty acid etc.

Instrumentation:

The GC Unit consists of Flowing mobile phase (inert gas - Ar, Ne, N), Injection port, separation column and detector; most common stationary phase for FAME analysis is DEGS (Diethylene glycol succinate). Mass spectrometry is the type of detector which separates ions based on their mass to charge (m/z) ratio and enables identification of fatty acids. In MS, the fatty acid is exposed to high energy electron that is broken down into charged fragments. The uniqueness of this process of fragmentation enables detection and identification of unknown fatty acids present in the sample.

Procedure for Fatty acid profiling using GC MS

Lipids are hydrolyzed in alkaline medium to extract the unsaponifiable material (sterol, alcohol, hydrocarbons, pigments, vitamins) present in them. For GC analysis, it is essential to convert these fatty acids into their methyl esters which are more volatile than the free fatty acid components.

Materials required:

1. Solvents : Chloroform, methanol, hexane, toluene, diethyl ether
2. 0.85% sodium chloride : Dissolve 0.85g of NaCl in 100 ml of distilled water
3. 1% sulfuric acid in methanol : Dissolve 1ml of conc sulfuric acid in 100ml of methanol
4. 25% sodium chloride : Dissolve 25g of NaCl in 100ml of distilled water
5. 2% Potassium bicarbonate : Dissolve 2g of potassium bicarbonate in 100ml of distilled water
6. 0.5N methanolic NaOH : Dissolve 2g of NaOH in 100ml of methanol
7. Saturated sodium chloride : Dissolve 25g of NaCl in 100ml of distilled water
8. Anhydrous sodium sulphite
9. BF3 – Methanol
10. Hexane
11. Fatty acid standard : Sigma FAME mixture (C4 – C24)

Sample extraction:

Excise the heart and rinse with ice-cold NaCl solution (0.9%). Store the samples in aluminum canisters at −20°C until laboratorial processing.

Extraction of lipid by Folch's method (Folch et al., 1957)

- Homogenize 5g heart tissue sample with 30ml of chloroform: methanol (2:1).
- Filter the homogenized mixture into a separating funnel and wait for 10 mins.
- Add 5ml 0.85%NACL into the separating funnel and shake well.
- Allow to stand overnight.
- Take initial weight of a completely dried round bottom flask
- Transfer the bottom chloroform layer into the pre weighed round bottom flask.
- Evaporate chloroform under vacuum at 50°C using rotary evaporator
- Again note the final weight of round bottom flask with sample

Preparation of FAME (Ichihara et al., 1996)

- Fix the flask containing sample into a FAME apparatus.
- Heating mantle should be adjusted to 40 ^{0}C and switch on the water connection
- Add 0.5N methanolic NaOH into the flask; wait for 5 mins
- Add 2ml BF3 for esterification; wait for 2 mins.
- Add 4ml n-heptane; wait for 8 mins
- Switch off the heating mantle; wait for 5 mins for complete condensation.
- Transfer the sample into a small round bottom flask
- Add saturated NaCl up to the neck of the flask
- Transfer the upper fat layer into a small vial containing small pinch of anhydrous sodium sulphite. Close the vial and store in refrigerator till GC analysis.

  5 µl of this sample will be injected through the injection port of GCMS and the chromatograms generated for the sample will be analyzed to identify the presence of fatty acids by considering its retention. Quantification is possible by determining the using the peak area of the chromatogram.

References

Maria Joao Lanc¸, Maria Machado, Catarina S. Mateus, Marta Lourenc, Ana F. Ferreira, Bernardo R. Quintella, Pedro R. Almeida (2014). Investigating Population Structure of Sea Lamprey (*Petromyzon marinus*, L.) in Western Iberian Peninsula Using Morphological Characters and Heart Fatty Acid Signature Analyses. PLoS ONE 9(9)

Hishikawa D, Shindou H, Kobayashi S, Nakanishi H, Taguchi R, et al. (2008) Discovery of a lysophospholipid acyltransferase family essential for membrane asymmetry and diversity. Proc Natl Acad Sci U.S.A. 105: 2830–2835.

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Ichihara K., Shibahara A., Yamamoto K., Nakayama T. 1996. An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 31: 535–539. [Erratum. 1996. *Lipids*31**:** 889].

Pavia, Donald L., Gary M. Lampman, George S. Kritz, Randall G. Engel (2006). Introduction to Organic Laboratory Techniques (4th Ed.). Thomson Brooks/Cole. pp. 797–817. ISBN 978-0-495-28069-9.

Proteomic tools in fish stock identification

Layana P, Tincy Varghese and Mohammed Ihzan

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Introduction

Conventional methods for fish species identification involves morphometric and meristic analysis based on the number of scales, scutes, fin rays, lateral line, calcified structures, life colour, body proportion etc. These methods cannot be considered as an effective tool as the characters are susceptible for changes due to ecological causes. Tools of identification based on biochemical genetic markers in fish such as electrophoretic protein profiling have been in use to solve taxonomic ambiguities that arises during morphometric analysis. Such genetic markers are superior to artificial markers as they are natural and can be found in all stages of animals. Muscle proteins (enzymes) are used as genetic markers which play a significant role in fishery biology and management. They are frequently used as valuable diagnostic characters in the classification of fishes at species, generic, familial and higher taxonomic levels. The difference in number of fractions, their mobility pattern I gel and staining intensity indicates species-specificity. Therefore, these protein fractions can be used as species -specific markers, which could be helpful in resolving disputes in the event of any taxonomic ambiguity.

Protein profiling by 2-Dimensional Gel Electrophoresis

Electrophoresis of proteins has been widely applied for direct study of genetic variation in fish population and identification of genetic stocks of commercially important fishery resources. Electrophoresis is defined as the process of migration of charged molecules through solutions in an applied electric field. The principle of electrophoresis is that a charged ion or group of ions in a suitable medium of electric field migrate towards one of the electrodes of opposite charge.

In 2D gel electrophoresis, the principle applied is that the proteins are resolved on a gel using isoelectric focusing (IEF), which separates proteins in the first dimension according to their isoelectric point, followed by electrophoresis in a second dimension in the presence of sodium dodecyl sulfate (SDS), which separates proteins according to their molecular mass. One advantage of 2D-PAGE as a separation technique is it not only resolves large numbers of proteins, but staining these proteins enables the relative abundances of the proteins to be quantified. The protein electrophoresis, especially the IEF of the sarcoplasmic proteins, is a

well-established technique for species identification of raw fish by analysing sarcoplasmic proteins in muscle. The proteins are characterized and separated by determining their isoelectric pH. Proteins migrate to a point in the gel corresponding to their isoelectric point and settles there creating a species specific band pattern. In SDS PAGE, the proteins have the ability to move when subjected to an electric field. The migration velocity is proportional to the ratio between the charges of the protein and it's mass. The higher the charge per unit of mass, faster the migration.

Procedure

Sample preparation

Sarcoplasmic protein extraction is done using double distilled water and 0.2M sucrose medium. Homogenize well. Transfer the sample to Eppendorf tubes and centrifuged at 4 ^{0}C at 10000 rpm for 30 mnt. Store the supernatant at -20 ^{0}C for later electrophoresis. (Bindhu V.,1998)

IEF

IEF is usually carried out at pH range of 3.0 to 10 using dried immobilized pH gradient (IPG strips). Strips are rehydrated using reswelling buffer. The soluble protein extracts of known concentration dissolved in IPG buffer is exposed to IEF on IPG strips using the electrophoretic apparatus. The IPGs are then equilibrated using equilibration solution 1 and 11. Finally the strips are placed on the top of resolving gel for second dimensional SDS-PAGE.

SDS-PAGE reagents

- Protein molecular weight standards
- 30% acrylamide -0.8% bis acrylamide
- Sample buffer: Mix 4 ml of 10 % SDS, 2 ml of glycerol, 1ml of βmercaptoethanol, 2.5 ml of 0.5 M tris-HCl (pH 6.8) and 0.03 g bromophenol blue.
- Bring the volume to 10 ml with distilled water prior to filter with Whatman No.
- 1 filter paper. Divide into 1 ml aliquots and stored at -200C.
- 10% (w/v) Ammonium persulfate)
- 10% (w/v) SDS
- TEMED (N,N,N',N'-tetramethylethlenediamine)
- 0.5M Tris-HCl, pH 6.8
- 1.5M Tris-HCl, pH 8.8

Electrode buffer: Dissolve 3 g of tris, 14.4 g of glycine and 1 g of SDS in distilled water. Adjust to pH 8.3. Add distilled water to 1 liter to total volume.

Staining solution: dissolve 0.05 g of comassie blue R-250 in 15 ml of methanol. Add 5 ml of glacial acetic acid and 80 ml of distilled water.

Destaining solution I: Mix 200 ml of methanol, 30 ml of acetic acid and 170 ml of distilled water.

Destaining solution II: Mix 50 ml of methanol, 75 ml of acetic acid and 875 ml of distilled water.

Table 2. Experimental set up for separating gel and stacking gel

Reagents	Separating gel		Stacking gel	
	7.5%	10%	15%	4%
30% acrylamide – Bis	2.5 mL	3.333ml	5.00mL	0.667 mL
1.5 M Tris HCl buffer pH 8.8	2.5 mL	2.5 mL	2.5 mL	---
0.5 M Tris HCl buffer, pH 6.8	----	----	----	- 1.25mL
Distilled water	4.845 mL	4.012 mL	2.345 mL	3.005 mL
10% SDS	100 µL	100 µL	100 µL	50 µL
10% Ammonium persulfate	50 µL	50 µL	50 µL	25 µL
TEMED	5 µL	5 µL	5 µL	3µL
Total	10 mL	10 mL	10 mL	5 mL

Method

Pouring the running gel

- Assemble the minigel apparatus according to the manufacturer‘s detailed
- Instructions. Make sure that the glass and the other components are clean and
- Dry before the assembly.
- Mix the separating gel solution by adding as defined in the table given below.
- Transfer 3.5ml of separating gel solution to the centre of the sandwich.

- Cover the top of the gel with a layer of distilled water. Allow the resolving gel
- To polymerise fully. (usually 30-60 min)

Pouring the stacking gel

- Pour off completely the layer of the distilled water.
- Prepare a 4% stacking gel solution by adding as described in the table given below.
- Transfer the stacking gel solution to tickle into the centre of the sandwich along an edge of one of the spacers.
- Insert comb into the layer of stacking gel solution by placing one corner of the comb into the gel slowly by lowering the other corner in. Allow the stacking gel to polymerize for 45 min at room temperature.

Loading the gel

- Dilute the protein to be 1:1(v/v) with the sample buffer in micro centrifuge tube and boil for 3 min at 1000C (boiling water)
- Remove the comb without tearing the edge of polyacrylamide wells.
- Fill the wells with electrode buffer.
- Place the upper chamber over the sandwich and lock the upper buffer chamber to the sandwich. Pour electrode buffer into the lower buffering chamber. Place the sandwich attached to the upper buffer chamber into the lower chamber.
- Fill the upper buffer chamber with the electrode buffer so that the sample wells of the stacking gel are filled with the buffer.
- Use a 10-25 μL syringe with a flat tipped needle; load the protein sample into the wells by carefully applying the sample as a thin layer at the bottom of the well
- Fill the remainder of the upper buffer chamber with additional electrode buffer.

Running the gel:

- Connect the power supply to the anode and cathode of the gel apparatus and run at a constant current of 15Ma / gel.
- After the bromophenol blue tracking dye has reached the bottom of the separating gel, disconnect the power supply.

Disassembling the gel

- Remove the upper buffer chamber and attached sandwich.
- Orient the gel so that the order of the sample well is known, remove the sandwich from the upper buffer chamber, and lay the sandwich on a sheet of absorbent paper or paper towels. Carefully slide the spacers out rom the edge of the sandwich along its entire length.

- Insert a spatula between the glass plates at one corner where the spacer was, and gently pry the two plates apart.
- Remove the gel from the lower plate.

Staining the gel

- Place the gel in a small plastic box and cover with the staining solution. Agitate slowly for 3 h or more on a rotary rocker
- Pour off the staining solution and cover the gel with a solution of destaining solution.
- Agitate slowly for about 15 min.
- For 2D spots staining usually colloidal staining is applied using Coomassie- blue G 250
- Pour off the destaining solution I and then destain with destaining solution II until the gel is clear except for protein bands.
- The protein bands obtained can be identified for its molecular weight or protein types by comparing with standard protein markers with known molecular weight and protein types.
- The band patterns were observed in a transilluminator over visible light

Reference

Block RJ, Durrum EL, Zweig G (2016). A manual of paper chromatography and paper electrophoresis. Elsevier.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Magdeldin S (2012). Gel Electrophoresis-Principles and Basics. InTech: Rijeka, Croatia, 1. Robyt JF and White BJ (1990). Biochemical techniques: theory and practice (Vol. 2).

Over view of Internal Ear and their function

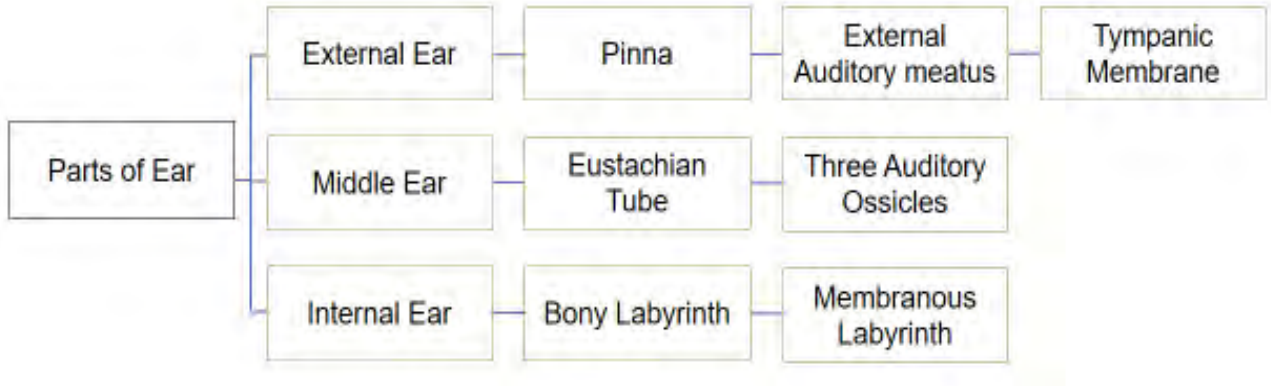
Vikas Pathak, Pawan Kumar and Shashi Bhushan

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Structure of Ear

The ear is consist of three parts as External ear, middle ear and internal ear. Pinna, External auditory canal (meatus) and tympanic membrane these are three parts of external ear, Eustachian tube and three ear ossicles together form middle ear bony labyrinth and membranous labyrinth these two parts together constitute internal ear (flow chart 1.)

Internal ears evolved first and occur in all vertebrates from Pisces to Mammals. Then evolved the middle ears that occur from Amphibians to Mammals but not in Pisces and external ears evolved in last that occur in Mammals only. It means all those animals who have pinna and external ears are mammals but exceptions are there Aquatic Mammals do not have external ears like Whale, dolphin and porpoise are some of the aquatic Mammals.



Flow chart 1.

Internal ear consists of two main divisions spiral shaped cochlea and vestibular apparatus. There are two window like structures in the internal ear, the oval shaped window called oval window and circle shaped window called circular window, internal ear communicates with middle ear with these two windows (fig. 1).

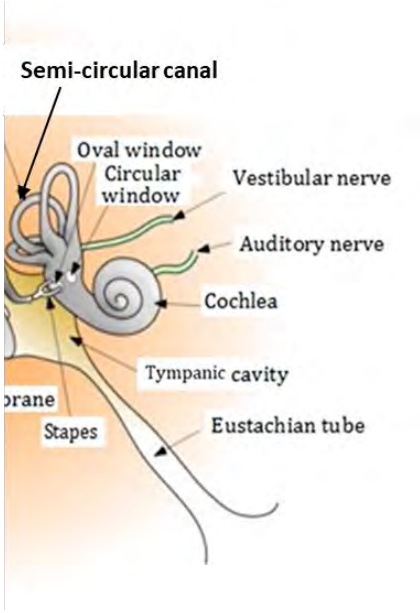


Fig. (1.a.) Structure of Ear

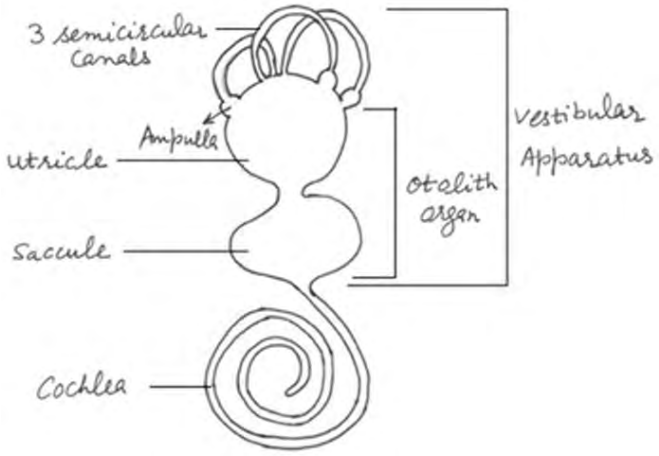
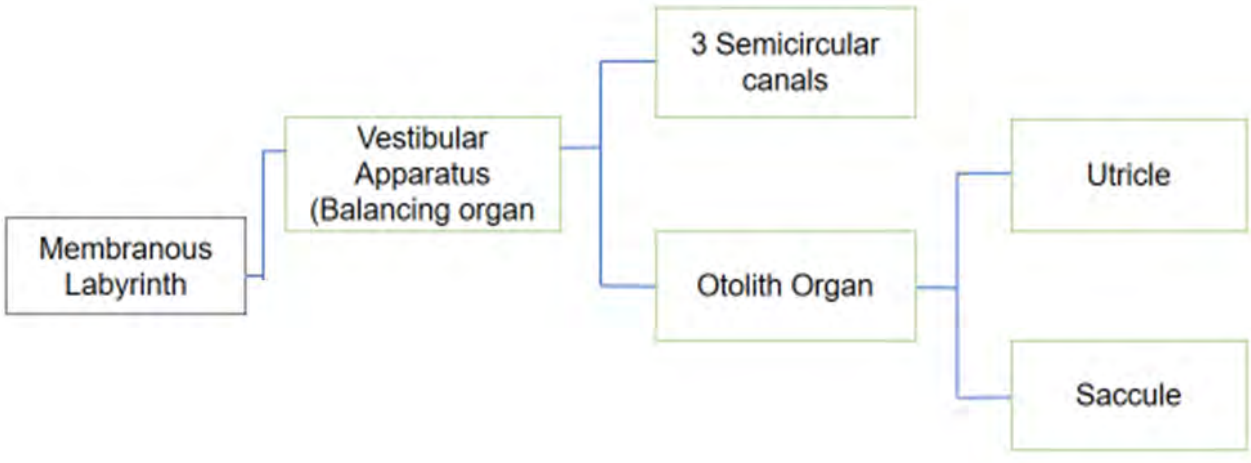


Fig. (1.b.) Hypothetical fig. of middle ear

Internal ear

The internal ear consists of two components bony labyrinth and membranous labyrinth (flow chart 2.). Bony labyrinth is a series of bony cavities in the temporal bone of neurocranium. Highly delicate structure called membranous labyrinth lies in the cavities of the bony labyrinth. Labyrinth means mess, internal ear is highly complicated structure that's why it call labyrinth (fig. 1b.)



Flow chart 2.

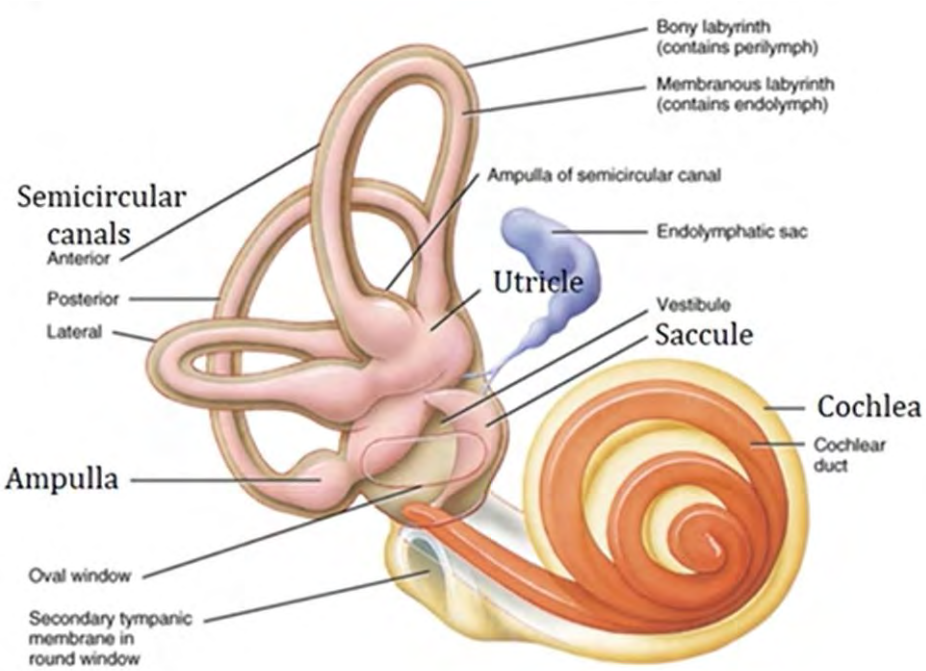


Fig. 2. Structure of Inner Ear

The all three semi-circular canals and the otolith organ called vestibular Apparatus (fig. 1b), all these semi-circular canals are situated at right angle to each other, one end of each semi-circular canal is swollen and this swollen end is called ampulla (fig.2). Semicircular canals are connected to utricle whereas cochlea is connected to saccule. The utricle and saccule and Cochlea both together called otolith organ (fig. 1b). Cochlea is a coiled structure and when uncoil this coiled structure, then stretched tube like structure appears and the longitudinal sections appear look like fig. 3, the oval window and the round window both are components of internal ear.

Vestibule

The vestibule is the outer bony part called labyrinth. This is composed of a specific type of lymph called perilymph which is rich in sodium, low in potassium. The outer bony labyrinth contains inside of it an inner membranous labyrinth consisting of a specific type of structures called saccule and utricle.

The saccule and the utricle consisting of a confluent structure called endolymph is high in potassium and low in sodium content whereas vestibule which is the outer bony labyrinth is called perilymph is low in potassium and high in sodium concentration.

The inner membranous labyrinth (endolymph) contains another specialized detector or sensory epithelium called the maculae. On the floor of the utricle maculae (stereocilia with hair cells) pointing up while the maculae of saccule pointing out.

Different types of equilibrium

Linear acceleration within the horizontal plane and tilting of body to the side, stimulate the hair cells that to be respond from the utricle and linear acceleration in the vertical direction stimulate the hair cells that to be respond from the saccule. Acceleration in upward direction leads hair cells down and deceleration leads hair cells up.

Longitudinal section of cochlea

There are three tube like structure appears in cochlea, upper most tube like structure called scala vestibule, the middle one is called scala media and the lower one is known as scala tympani. There are two types of fluids scala vestibuli and scala tympani filled with perilymph whereas scala media is filled with endolymph. there is narrow passage like structure at the end of cochlea known as helicotrema that connects both tubes (Scala vestibuli and scala tympani), the membranes found between these tubes like Reissner's membrane lies between scala vestibule and scala media (fig.3.) whereas basilar membrane separates scala media from scala tympani, an Organ of Corti rests on the basilar membrane are the hair cells which are in the contact (by stereo cilia) with the tectorial membrane (fig.4)

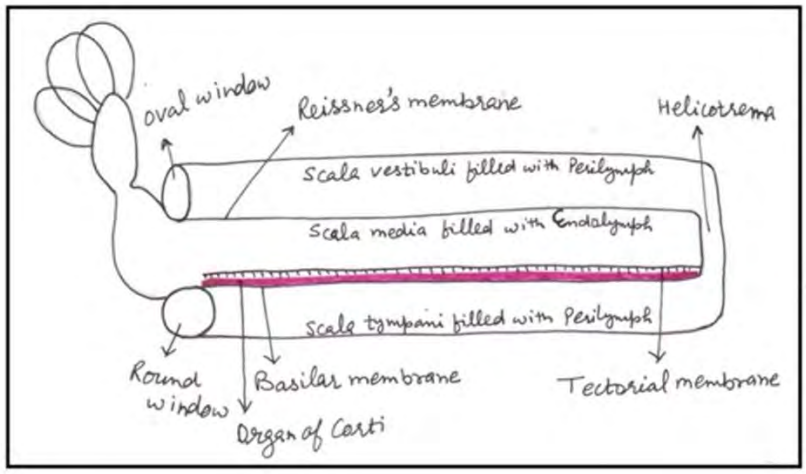


Fig. 3. Longitudinal section of stretched cohlea

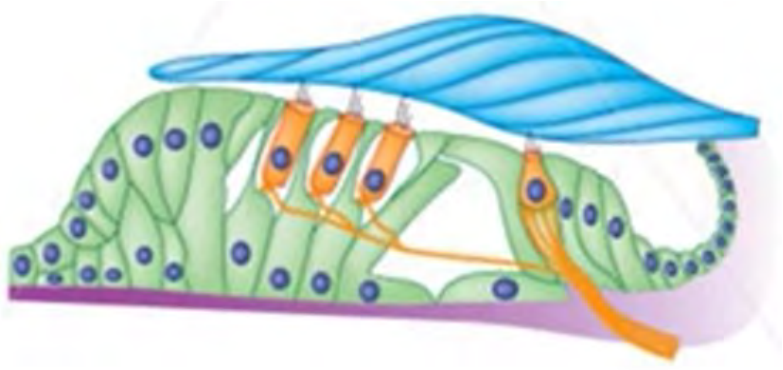


Fig. 4. Longitudinal section of cochlea

Transverse section of cohlea

In the transverse section of cochlea there are three cavities, upper most cavity is scala vestibule, middle one is scala media and the lower most is called scala tympani. Reissner's membrane lies between scala vestibule and scala media whereas basilar membrane separates scala media from scala tympani. A hearing organ is found on the basilar membrane that is called organ of corti, the tectorial membrane found just above the organ of corti (fig. 5).

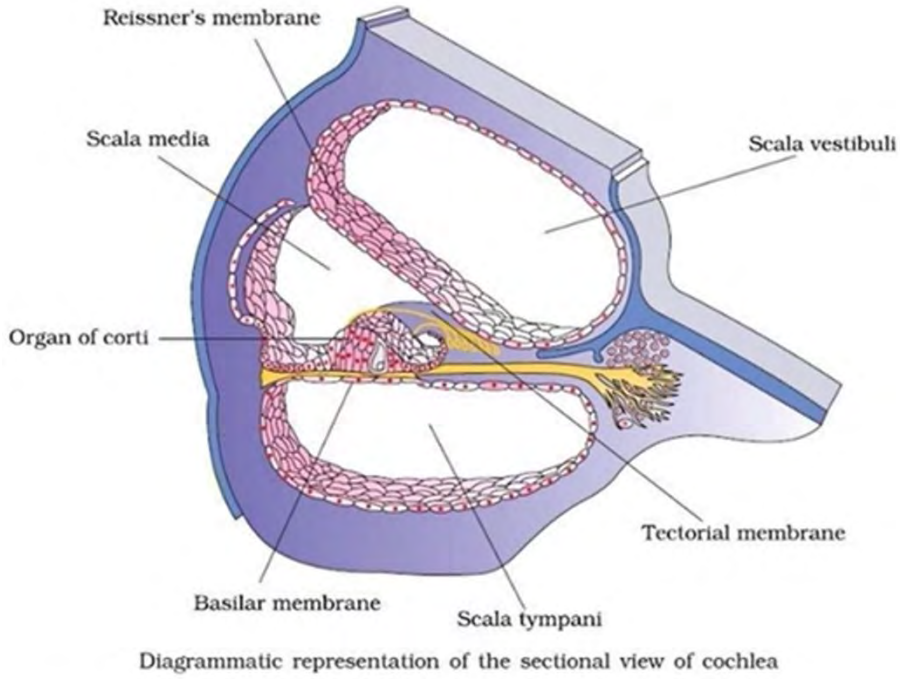


Fig. 5. Transverse section of cohlea

Mechanism of Hearing

The sound waves shown in the diagram (fig.6) which hit the tympanic membrane and generate the vibrations in the tympanic membrane and these vibrations reach to oval window through ear ossicles and oval window starts vibrating and these vibrations proceed ahead and reach to perilymph of the scala vestibuli as a result perilymph start vibrating and these vibrations picked up by Reissner's membrane, these vibration proceed ahead and reaches to scala media and the endolymph start vibrating when endolymph vibrates, the stereocilia of the hair cells start rubbing against the tectorial membrane the stereo cilia rubbing against the tectorial membrane due to vibrations in the endolymph then due to action potential generation the impulse is generated and this nerve impulse reaches to a particular region in brain through cochlear or auditory nerve and the brain analyses the impulse and provide output accordingly.

The vibrations are generated in the fluids which create a kind of pressure in the fluids of the internal ear. Releasing of pressure from fluid is very important otherwise pressure keep

on increasing may cause damage of internal ear. The pressure releases vibration which are generated in the perilymph of the scala vestibuli and these vibrations proceed to reach perilymph of the scala tympani where these vibrations hit against the round window and generate vibrations in the round window and release the pressure. The pressure generated in the fluids is released by hitting against the round window so this is mechanism of hearing (fig. 6).

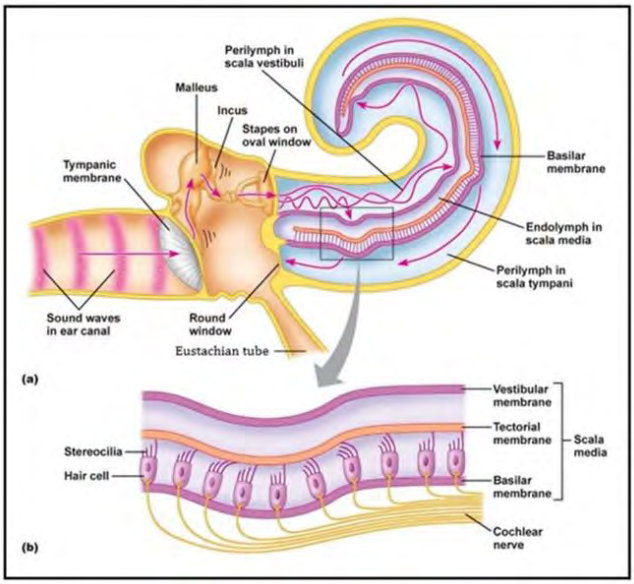


Fig. 6. Mechanism of Hearing

Reference

McN. Alexander R., –The Structure of the Weberian Apparatus in the Cyprini," Proc. Zool. Soc. (London) 139, 451—473 (1962).

McN. Alexander R., –Physical Aspects of Swimbladder Function," Biol. Rev. 41, 141– 176 (1966).

Amoser S. and F. Ladich, –Diversity in Noise-Induced Temporary Hearing Loss in Oto[hysine Fishes," J. Acoust. Soc. Am. 113, 2170–2179 (2003).

Bigelow H., –The Sense of Hearing in the Goldfish *Carassius auratus*", Amer. Natur. 38, 275- 284 (1904).

Blackmann H., U. Niemann, and B. Fritzsch, –Peripheral and Central Aspects of the Acoustic and Lateral Line System of a Bottom Dwelling Catfish, *Ancistrus* sp.," J. Comp. Neurol. 314, 452–466 (1991).

Blaxter J. H. S., –Structure and Development of the Lateral Line," Biol. Rev. 62 (4), 471–514 (1987).

Blaxter J. H. S. and R. S. Batty, –The Development of Startle Responses in Herring Larvae," J. Mar. Biol. Assoc. U.K. 70, 737–750 (1985).

Von Frisch K. and H. Stetter, –Unersuchungen über den Sitz des Gehörsinnes bei der Elritze," Z. Vergl. Physiol. 17, 686–801 (1932).

Froloff J., –Bedingte Reflexe bei Fischen. I.," Pflüger Arch. Ges. Physiol. 208, 261– 271 (1925).

The Otolith: Features and Function

Vikas Pathak, Shashi Bhushan, Layana P. and Pawan Kumar

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Introduction

Otoliths (–earstones") are small, white structures found in the head of all fishes other than sharks, rays, and lampreys. Although they are located within the skull adjacent to the brain, they are not attached to the skull, but are retained within the transparent tubular canals of the inner ear. Otoliths provide a sense of balance to fish in much the same way that the inner ear provides balance in humans. Fish otoliths also aid in hearing. To the fisheries biologist, the otolith is one of the most important tools for understanding the life of fish and fish populations.

Growth rings (annuli) not unlike those of a tree record the age and growth of a fish from the date of hatch to the time of death. Daily growth increments formed in the first year of life record daily age and growth patterns in surprising, albeit microscopic, detail. In addition, chemical and elemental assay sallow the reconstruction of everything from the year of hatch, to migration pathways, to population identity, to the temperature of the water.

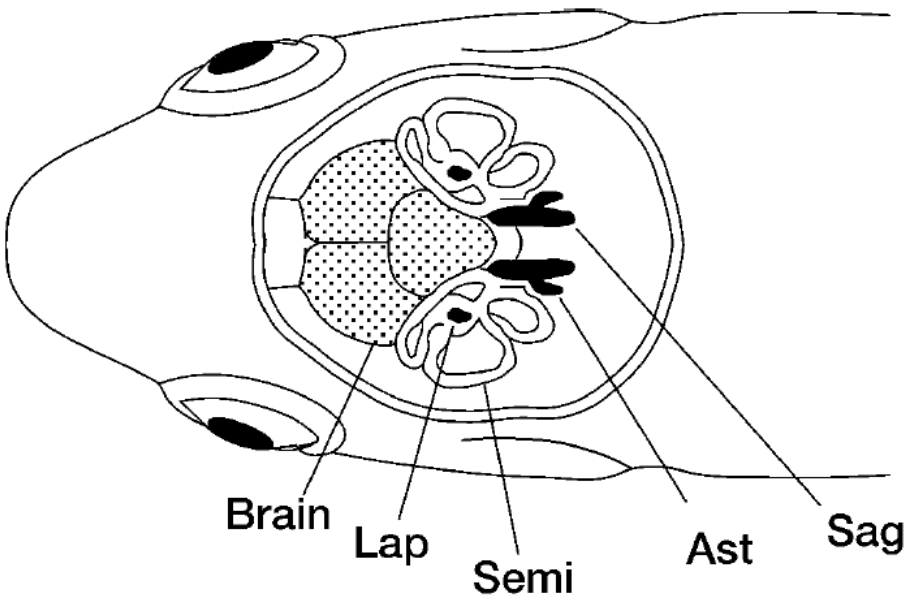
Indeed, virtually the entire lifetime of the fish is recorded in the otolith. For that reason, otoliths are used and studied in almost every fisheries laboratory in the world, and form the basis for most age-structured analyses of fish populations around the world. Recent estimates indicate that more than 800 000 otoliths were aged worldwide till 1999, with many more analysed for shape, chemical composition, and other applications. Otoliths have a distinctive shape which is highly species specific, but varies widely among species. Thus fish, seal, and seabird biologists, as well as taxonomists and archaeologists, often rely on the shape and size of preserved or undigested otoliths to reconstruct the species and size composition of the diet of fish predators. Preserved otoliths may also serve to identify fossil fish assemblages for phylogenetic or climatological studies.

Identification is aided by the fact that otoliths resist degradation better than most other tissues, and are often the only identifiable animal remains recovered from stomachs and droppings, as well as from Indian middens. Reference collections of otoliths now exist for several locations around the world, although none claim to be comprehensive.

Otolith location and function

The inner ear is the primary vestibular organ in fishes and other vertebrates, responsible for balance and orientation in three dimensions. The inner ear also aids in sound detection in most fishes. The inner ear is located adjacent to the brain, within and occasionally invaginated in the neuro-cranium (Fig. 1). Composed of a series of inter connecting semi-circular canals, the fluid-filled inner ear looks delicate and translucent but is surprisingly tough. Inner ear architecture varies somewhat among species, but a common feature is the presence of three pairs of chambers, each of which contains an otolith (Fig. 1). Since otoliths are the only solid bodies within the endolymph fluid, changes in orientation and acceleration are detected by slight shifts in the location of the otoliths relative to the surrounding chamber. A sensory epithelium in the form of a macula lies on one wall of the chamber, coupled to the otolith via an otolithic membrane.

This sensory epithelium is thought to be responsible for the detection of both sound and changes in posture. Thus all otoliths appear to share a vestibular and sound detection function, although the balance between the two functions may vary with the otolith. In general, the lapilli appear to be more associated with posture and the sagittae with sound detection.



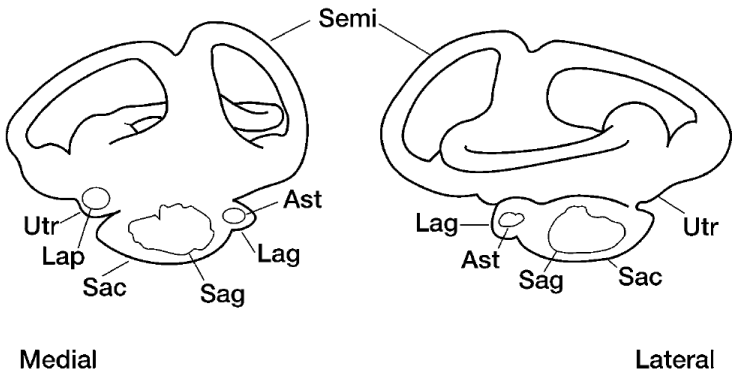


Fig. 1. Schematic of the location of the inner ear and three pairs of otoliths in the skull of a generalized teleost

The three pairs of otoliths tend to have a size and shape representative of the chamber within which they are held (Fig. 1). Each sacculus contains a sagitta (plural: sagittae), which is often the largest otolith in all but the ostariophysian fishes. The sacculus and sagitta on a given side are usually ventral to the posterior part of the brain, lateral to but close to the midline of the brain. Slightly posterior to each sacculus is a lagena, containing an asteriscus (plural: asteriscii). The asteriscus is so close to the sagitta in many fishes that they are often removed together when the sacculus is pulled out. Considerably more anterior and dorsal on each side is the utriculus containing the lapillus (plural: lapilli). The lapillus is often the smallest of the otoliths. In ostariophysian (otophysan) fishes, a chain of Weberian ossicles connecting the swimbladder to the inner ear enhances sound detection. In these fishes, the asteriscii are usually larger than the sagittae.

Otolith composition

Otoliths are very pure compared to most biological and mineralogical structures, with the composition being dominated by calcium carbonate in an organic matrix. Most otoliths contain more than 95% by weight of calcium carbonate, with 3 –5% in the form of an organic matrix, and less than 1% as non-organic trace impurities. The trace element and stable isotope composition of the otolith has been given extensive study, owing to numerous applications in reconstructing the environmental history, migration, and population identity of fishes.

Calcium carbonate can crystallize as any one of three crystal polymorphs: calcite, aragonite, or vaterite. However, the vast majority of sagittal and lapillar otoliths are composed of aragonite, which has a milky white appearance. This is unlike the otoconia of mammals, which are composed of calcite curiously, different polymorphs of calcium carbonate appear to be linked to the different otolith organs. While aragonite is the norm for sagittae and lapilli, most asteriscii are made of vaterite, thus accounting for their glassy

appearance. Vaterite is also the principal polymorph in many aberrant, or –erystalline", otoliths. Calcitic regions in otoliths are much rarer.

The implications of an otolith composition dominated by calcium carbonate lie most clearly with otolith preservation and stability. Both calcium carbonate and otoliths are stable for many years when stored dry. However, calcium carbonate is acid soluble, so preservation in even weakly acidic solutions will result in dissolution of the otolith.

Otolith morphology

The three pairs of otoliths differ markedly in shape and appearance. In most adult fishes, the sagittae are the largest pair and the lapilli the smallest. In contrast, as teriscii are larger than sagittae in ostariophysian fishes (a group which includes the minnows and catfish). Sagittal shape differs substantially among species, while lapillar shape is more uniform. The shape of the asteriscii shows intermediate inter-specific variability. Within an otolith pair, the left and right otoliths are very similar, but not identical, mirror images of each other. Interestingly, the left and right asteriscii can differ considerably more in shape than the other otolith pairs.

The orientation and major landmarks of a typical sagitta are shown in the Fig. 2. The rostrum, anti-rostrum, and post-rostrum are consistent features of all sagittae, although their size and extension varies substantially among species. The sulcus, which represents the point of attachment of the sensory macula, is also a consistent feature of sagittae. It is possible that inter-specific differences in the shape of asteriscii and lapilli could be used to complement the differences observed in the sagittae. In virtually all young fish larvae, otoliths tend to be relatively featureless: spherical or smoothly oblate in most species, and discoid in species (such as salmonids) which hatch at a larger size.

In most species, the sagittae and lapilli are present at hatch, while the asteriscii first appear at an age of 2–3 weeks. At this early stage of fish development, relative otolith sizes can be inverted, with lapilli being larger than sagittae. Otoliths first acquire the main features of their mature shape in the juvenile stage.

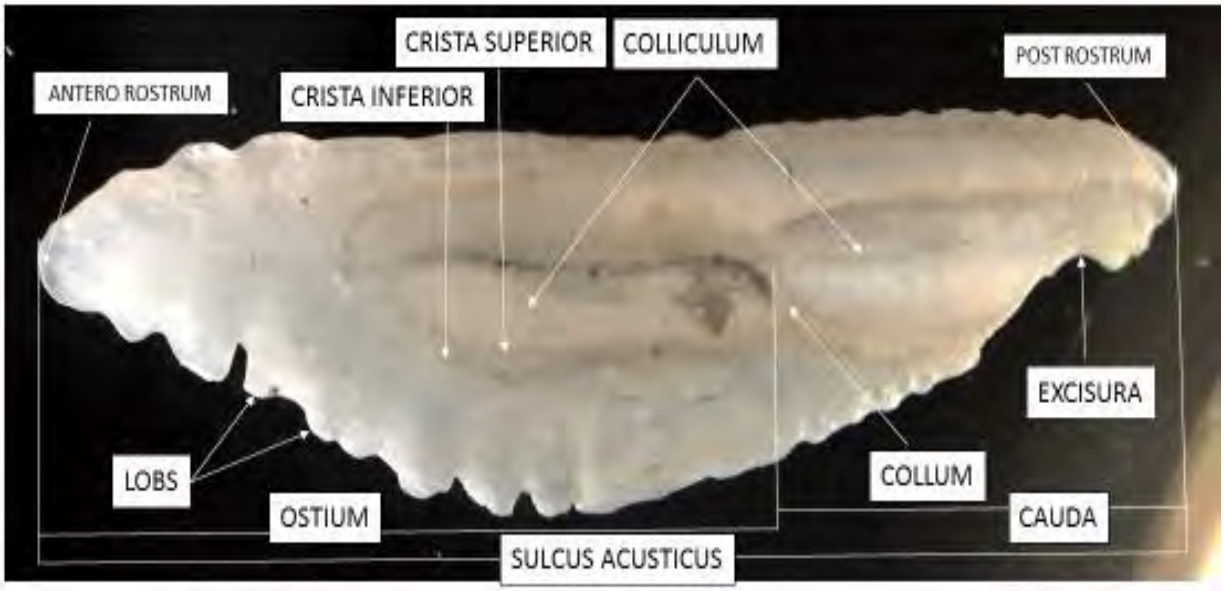


Fig. 2. Major landmarks of a typical sagitta

The otolith shape of very large fish can differ substantially from those of average-sized adults. Because of their function in maintaining the balance of the fish, otoliths tend to grow as the fish grows. Therefore, there is almost always a strong relationship between otolith size and fish size. It is also important to note that the relationship between fish and otolith length is not necessarily linear, and that the relationship for larvae is often very different from that for adults.

Biological factors affecting otolith morphology

There are a broad range of biological factors which influence or moderate otolith shape. These factors can operate at a range of scales, from that of general phylogeny to the individual level. Few of these factors are well understood, but those that are known or suspected are mentioned here. There are no broad phylogenetic principles which are known to guide otolith shape. Although family-or genus-level otolith characteristics are often present, it is often impossible to predict otolith shape for any given species. There may be functional relationships however. Based on numerous observations, it have been noted that fast-swimming fishes capable of rapid acceleration and turning tend to have smaller otoliths than their slower swimming counterparts.

The tunas and swordfish are good examples of this phenomenon, whereby the sagittal otolith of a 400-kg blue fin tuna (*Thunnus thynnus*) is smaller than that of a 1-kg cod. Species capable of good sound production (and presumably good sound detection) can also be expected to have large saggital otoliths.

Members of the Sciaenidae (grunts and drums) are characteristic of this group; species such as the black drum (*Pogonias cromis*) have sagittae which are among the largest observed. In contrast, the families within the group Ostariophysii (such as cyprinids and catfishes), which possess a chain of Weberian ossicles to enhance sound detection, have somewhat smaller sagittae and larger asteriscii than normal. Within a species, otolith shape can vary with the sex, population, and growth rate, as well as the stage of ontogeny described in the previous section. The magnitude of shape differences due to ontogeny and fish size is considerably larger than that due to sex, population, and growth rate, since the effect of the latter factors may be detectable only through statistical analysis.

In general, otoliths within an otolith pair are very similar but non-identical mirror images of each other. However, left versus right asymmetry is common in flatfish, a fish in which the eyes migrate to the same side of the head at around the time of metamorphosis to the settled juvenile. The presence or degree of asymmetry seems to vary among individuals, and is most evident in large individuals. In general, however, the sagitta found on the upper side of the fish (the right otolith in right-eyed flatfish) is irregularly shaped or occasionally shorter and thicker than the sagitta which faces down in the adult fish. The functional significance of this otolith asymmetry is unknown, but is presumably related to a reduced or altered function in one of the two otoliths.

Effects of preservation on otolith morphology

The shape and size of otoliths recovered from the stomach of fish predators has long been used to reconstruct the species and size composition of the predator's diet. Several studies have fed fish of known species and size composition to seals, and then recovered the ingested otoliths from the stomachs. In all cases, sources of bias have been noted, associated primarily with the relatively rapid dissolution of small and (or) fragile otoliths in the acidic stomach environment. As a result, it seems likely that any dietary reconstruction could underestimate the contribution from fish species with small otoliths, or from smaller individuals. Even where complete otolith dissolution does not occur, partial dissolution can leave an otolith unrecognizable to species, or perhaps smaller than its original size.

Otoliths may be recovered from aquatic sediments, fossil grounds, or archaeological middens, where they have been exposed to possibly acidic conditions or chemical leaching. Such conditions have the potential to bias reconstructions of past assemblages, if otoliths have been dissolved, or to alter climatic reconstructions if chemical leaching has occurred. However, other indicators can often be used to determine if otolith alteration has occurred. In the case of archaeological applications, bias is not usually a problem, since the growth

increments used to determine seasonality are either present or absent. For reasons not fully understood, otoliths in some fossil middens may sustain little damage after thousands of years of preservation, while others are rendered illegible after only a few hundred years.

Definition and descriptive terms of otolith

Antirostrum: An anterior dorsal extension of the otolith, above notch. Antiostrum is not found when no excisura major is present or extension is absent.

Cauda: Exterior part of sulcus, posterior margin to neck constriction

Colliculum: The embossed part of sulcus floor, differentaied into caudal colliculum and ostial colliculum, according to their location

Collum: The embossed part of sulcus floor or neck, dividing ostium and cauda.

Crista inferior: Ventral margin of sulcus.

Crista superior: Dorsal margin of sulcus.

Dorsal area: The region lying dorsal to sulcus.

Dorsal depression: Depression in the dorsal area.

Excisura (or excisura major): Opening of the sulcus on the front margin. If sulcus does not reach to the rim, there will not be excisura.

Neck: The colligation of ostium and cauda, it is generally formed in the width of sulcus.

Notch: The nick on the excisura major, dividing rostrum and antirostrum, if present.

Ostium: Frontal part of sulcus, prior to neck constriction

Pseudocolliculum: The collicular ridge above crista inferior.

Excisura minor (or pseudo-excisura): The opening of the sulcus on the posterior margin, only seen in otoliths with caudal sulcus.

Rostrum: An anterior most extension of the otolith, measured from the posterior margin of notch to anterior rim of the otolith. Rostrum is absent when excisura major is absent.

Sulcus: Depression on the middle face, generally longitudinal and located in the center. In maximum cases, it can be divided into ostium and cauda.

Ventral area: The surface that is lying on ventral to sulcus.

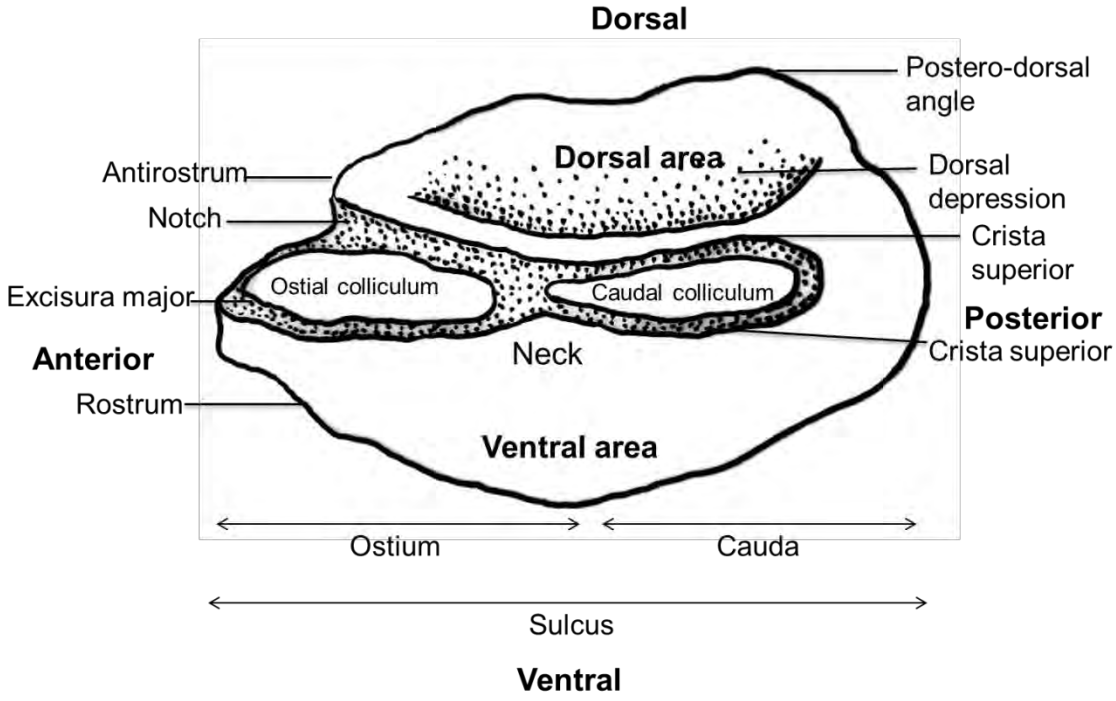


Fig. 1. Medial face of a generalized right otolith showing the positions of arious features.

Sulcus may typically open to one or more than one margins (Fig. 2). Cristae define the periphery of sulcus. If colliculum reaches the rim, then sulcus is well throught out to be open even if cristae are not visible. Definitions of the sulcus types are as follows:

Caudal: Sulcus opens to posterior or ventral periphery and ostium is closed.

Mesial: Sulcus has no marginal opening, in other term sulcus is closed.

Ostial: Sulcus opens to dorsal or anterior margin and cauda is closed.

Ostio-caudal: Sulcus opens to both anterior and posterior rim.

Ostio-pseudocaudal: Sulcus opens to dorsal margin or anterior and cauda lies about to ventral or posterior margin but it is still closed.

Para-ostial: Sulcus opening is reduced and a narrow canal present towards anterior or dorsal margin. Cauda is closed.

Pseudo-ostial: Ostium lies close to dorsal or anterior margin but it is still closed. Cauda is closed.

Pseudo-ostiocaudal: Sulcus present very near to anterior and posterior rim but it is still closed.

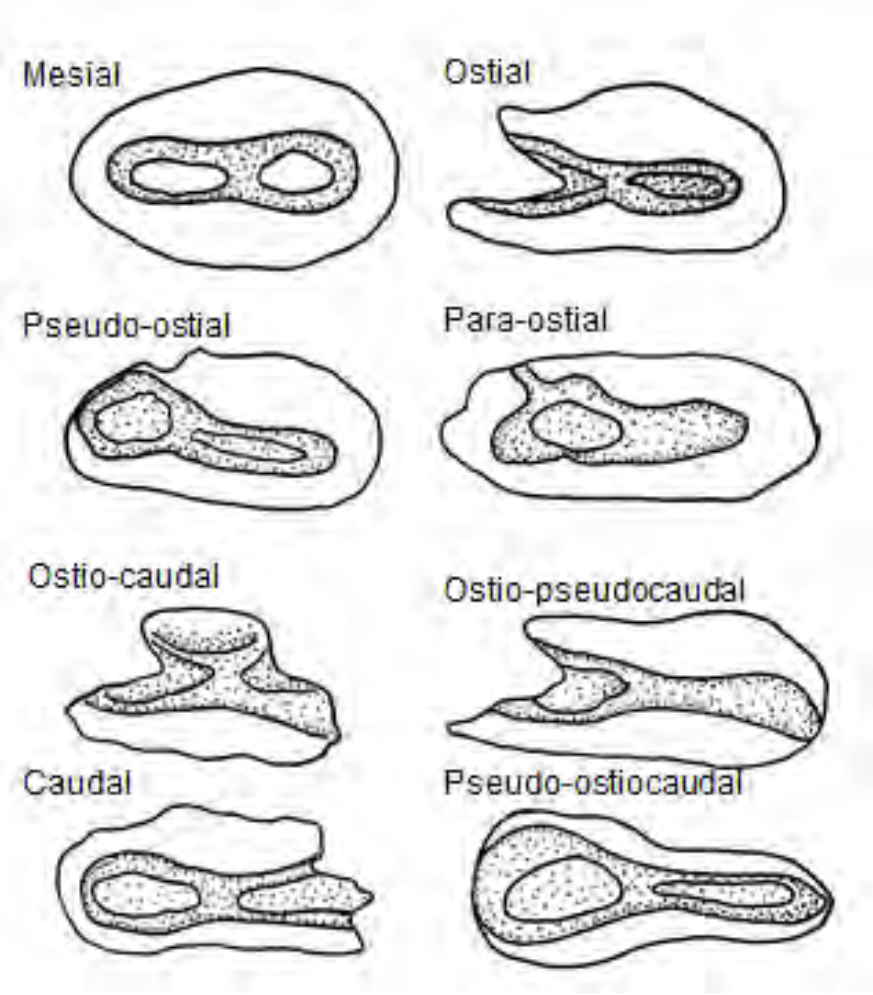


Fig. 2. Diagrams of otoliths with the corresponding terms according to sulcus openings

Collum can be divided into three types: wall-like, raised tubercle and solid bridge (Fig. 5).

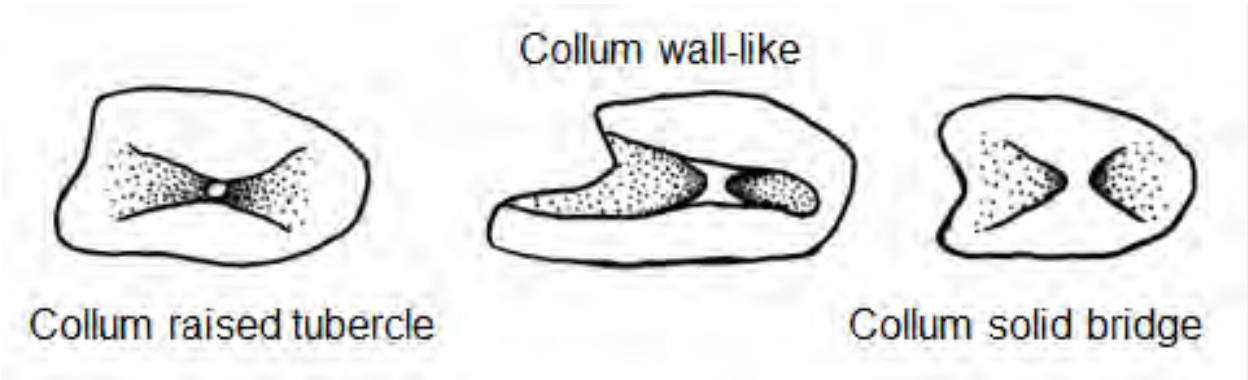


Fig. 3. Diagrams of otoliths with the corresponding terms according to collum types

Cristae (crista superior and crista inferior) are described as per their development: absent, poorly-developed, well-developed and ridge-like (Fig. 6).

Absent: The edge between sulcus and other parts of the middle surface is unclear.

Poorly-developed: The edge between sulcus and other parts of the middle surface is clear but cristae are not embossed.

Ridge-like: Cristae are embossed as ridges above other parts of the middle surface.

Well-developed: The edge between sulcus and other parts of the medial surface is clearly delimited; cristae are often slightly raised but not ridge-like.

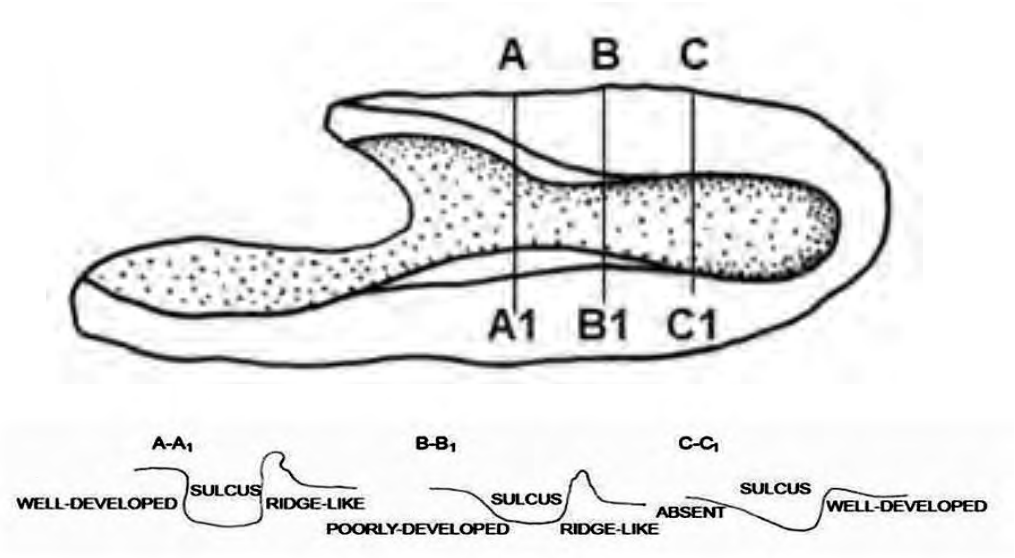


Fig. 4. Diagrams of otoliths with the corresponding terms according to cristae development with cross sectional views along axes.

Fig. 5. Images of otoliths with terms of otolith shapes described in this atlas.

Reference

Andrus, C.F., Crowe, D.E., Sandweiss, D.H., Reitz, E.J., and Romanek, C.S. 2002. Otolith record of mid-Holocene sea surface temperatures in Peru. Science, 295: 1508–1511.

Barrett, R.T., Rov, N., Loen, J., and Montevecchi, W.A. 1990. Diets of shags *Phalacrocorax aristotelis* and cormorants *P. carbo* in Norway and possible implications for gadoid stockrecruitment. Mar. Ecol. Prog. Ser. 66: 205–218.

Bigelow, H.B., and Schroeder, W.C. 1953. Fishes of the Gulf of Maine. U.S. Fish Wild l. Serv. Fish. Bull. 74. Vol. 53. 577 p. Bowen, W.D. 2000. Reconstruction of pinniped diets: accounting for complete digestion of otoliths and cephalopod beaks. Can. J. Fish. Aquat. Sci. 57: 898–905.

Elder, K.L., Jones, G.A., and Bolz, G. 1996. Distribution ofotoliths in surficial sediments of the U.S. Atlantic continentalshelf and slope and potential for reconstructing Holocene fish stocks. Paleoceanography, 11: 359–367.

Fossum, P., Kalish, J., and Moksness, E. 2000. 2nd International Symposium on Fish Otolith Research and Application, Bergen, Norway, 20–25 June 1998. Fish. Res. 46: 1–371.

Gaemers, P.A.M. 1984. Fish otoliths from the Bassevelde sand (Late Tongrian) of Ruisbroek, Belgium, and the stratigraphyof the early Oligocene of Belgium. Meded. Werkgr.Tert. K wart. Geol. 46: 237–267.

Hales, Jr., L.S., and Reitz, E.J. 1992. Historical changes in ageand growth of Atlantic croaker, *Micropogonias undulates* (Perciformes: Sciaenidae). J. Archaeol. Sci. 19: 73– 99.

Jobling, M., and Breiby, A. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia, 71: 265–274.

Maisey, J.G. 1987. Notes on the structure and phylogeny of vertebrate otoliths. Copeia, 2: 495–499.

Morrow, J.E. 1976. Preliminary keys to otoliths of some adultfishes of the Gulf of Alaska, Bering Sea, and Beaufort Sea. NOAA Tech. Rep. NMFS Circ. 420: 1– 32.

Nolf, D. 1985. Otolithi Piscium. *In* Handbook of Paleoichthylogy. Vol. 10. *Edited by* H. P. Schultze. Gustav Fischer Verlag, New York. 145 p.

Nolf, D. 1995. Studies on fossil otoliths the state of the art.*In* Recent Developments in Fish Otolith Research. *Editedby* D.H. Secor, J.M. Dean, and S.E. Campana. University of South Carolina Press, Columbia, SC. p. 513–544.

Oliveira, A.M., Farina, M., Ludka, I.P., and Kachar, B. 1996.Vaterite, calcite and aragonite in the otoliths of three speciesof piranha. Naturwissensch aften, 83: 133–135.

Pierce, G.J., Boyle, P.R., and Diack, J.S.W. 1991. Identification of fish otoliths and bones in faeces and digestive tracts of seals. J. Zool. (London), 224: 320–328.

Applications of Otolith

Vikas Pathak, Shashi Bhushan, and Karan K. Ramteke

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Introduction

The three pairs of otoliths are known to be the _sagittae', _lapilli' and _asterisci', and are each contained within individual vestibules. They form in the embryonic stages of the fish, grow continuously throughout its life, and are composed of alternating layers of calcium carbonate (usually in the mineral form aragonite) and protein, which are deposited on a daily basis. Otolith growth is continuous and is maintained even through periods when somatic growth is virtually non-existent. As they form, otoliths absorb elements from the ambient water, which vary in relation to environmental conditions, such as salinity and temperature.

Otolith formed by acellular mechanism that mean once the material in otoliths is deposited, it is generally not reworked or resorbed; otolith chemistry is thus a function of the environmental conditions experienced by the fish. This is a very important property of the otolith for palaeo-environmental and archaeological applications. Their chemical composition affords the possibility of environmental reconstruction that, when matched with otolith biochronologies, can allow the lifetime of an individual fish to be placed retrospectively within time and space.

A large array of data are able to be recovered from otoliths, including species identification, age and growth studies, seasonality, radiocarbon dating and trace element and isotope analysis. Otolith studies frequently contribute to answering questions focusing on changes in fish population structures, including examining impacts of intense human predation, environmental change and habitat destruction.

Otoliths also provide a wide range of information regarding the past occupants of a site; human subsistence strategies, fishing methods and technologies, trade routes, seasonality of site usage, and past human responses to environmental changes can all be examined through the analysis of these small carbonate structures few example have been described here.

Fish Size

Fish grow larger the longer they live, though growth capacity is dependent on both internal (nervous, endocrinological and neuroendocrinological) and external ecological factors (salinity, temperature, food) While somatic growth can be slowed or interrupted. The

otolith growth is continuous three-dimensional process, with the length, width and depth of an individual organism all changing over time. This relationship enables the size of a fish at the time of its death to be determined by analysing the weight(s) of its otolith(s). Similarly, otolith length can also be used.

It is important to note that that while there is a strong correlation between otolith size and fish size within species, specific relationships do not correspond between species. Small fish species do not necessarily have small otoliths, and large fish do not always have large otoliths.

The size of fish present in the archaeological record may be indicative of the fishing techniques that were employed by local Indigenous populations: spearing in shallow water usually results in the capture of larger specimens, as they are easier to hit; gill nets have a high degree of size selectivity, capturing a narrow size range of fish dependent on the net's mesh size; fish traps of stone, netting or wicker-work will catch all fish over a certain size; and hook and line fishing tends to catch predatory fish whose size can be dependent on the size of the hook. Past fishing methods can inform about the technological skills and knowledge of a society, and may indicate the relative importance of fish in the diet and community, based on the time and energy involved in fishing.

These studies suggested habitat alteration and intensive human predation as likely causes for the decreases in fish size and demonstrate that even basic analyses of fish otoliths can result in significant findings concerning past fish population structures. Some application of otolith are mentioned here.

Age Structure

The appearance of the aragonite on the organic matrix within the otoliths changes depending on physiological and environmental factors. These variations result in the formation of bands, or annuli, within the otolith's structure. They are defined by two zones; a slow growth zone, that, when viewed under a transmitted light source, appears as thin bands, darker in colour, and a fast-growth zone that appears as thick, lighter-coloured, or hyaline, bands. Aragonite and organic compounds are found in both zones, with greater concentrations of organic compounds in the fast growth zones, and greater concentrations of aragonite in the tight carbonate bandings of the slow growth zones. It is known that development and growth are influenced by both internal and external factors.

Edge Increment Analysis

In addition to providing an estimate of age of death of the fish, the annuli can also provide information about the season of death. In addition to providing an estimate of age of death of the fish, the annuli can also provide information about the season of death. Good seasonality estimations can be obtained when a large sample size can be analysed, and when studies of modern samples of the same species have been conducted to demonstrate when edge increments are laid down.

Trace Element Analysis

This is possible because concentrations of elements vary, and are influenced by salinity, temperature, ambient water chemistry. In otolith a total of 31 elements have been detected to date, not including radioactive elements such as Th and Ra. The elements are Ca, Na, Sr, K, S, Cl, P, Mg, Si, Zn, B, Fe, Hg, Mn, Ba, Ni, Cu, Al, Br, Li, Pb, As, Se, Ag, Co, Cd, U, Cs and major element C, O, N and trace radioactive elements Ra and Th (ascending order in composition by mass).

$$\text{Sr:Ca ratio} \quad \alpha \quad \text{Marine water W (salinity)}$$

$$\text{Sr:Ca ratio} \quad \alpha \quad \frac{1}{\text{Fresh water}}$$

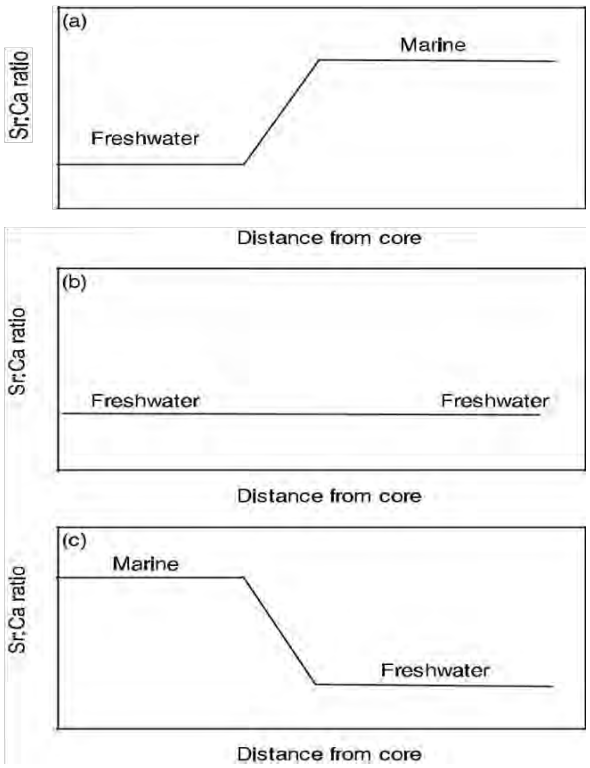
$$\text{Ba:Ca ratio} \quad \alpha \quad \frac{1}{\text{Marine water W (salinity)}}$$

The calcium/strontium ratio can be used in combination with increment number to estimate the dates of migration of anadromous and catadromous species. Other trace elements, such as K, Mn, Li, Mg and Ba have also proved their utility as natural tags of the nursery ground origins of juvenile fish and spacio–temporal distribution of stocks etc. the analysis of composition of element used to know the migration pattern as given below.

Catadromous

Potamodromous

Anadromous



Relationship between the element:Ca ratio and salinity and temperature is species specific. Temperature had a minor influence on otolith chemistry relative to salinity

Isotope Analysis

A range of stable isotopes have been analysed in modern fish otoliths, while the most widely used elements in isotopic studies of archaeological otoliths are oxygen (^{18}O) and carbon (^{13}C), with fewer studies focusing on nitrogen (^{15}N). These analyses are becoming increasingly popular and important for understanding past environmental and cultural changes.

Oxygen isotope (^{18}O) ratios in otoliths are determined primarily by water temperature and can consequently provide information on environmental change seasonality of site usage, fish location and migration. As water temperatures increase, the uptake of ^{18}O in otoliths decreases.

Oxygen isotopes are robust tracers of the marine stage of life history because large and systematic differences exist between marine and inland water isotope values, and the oxygen isotopic composition of fish otoliths depends upon the temperature, salinity and isotopic composition of the ambient water, not food. Despite the well-defined relationship between temperature and ^{18}O ratios, water salinity can also have an effect, while temperature and salinity can interact to influence ratios.

A progressive depletion of ^{18}O in rain and surface water occurs with increasing latitude, increasing elevation, and increasing distance inland from the ocean. The carbon in otolith aragonite is a mixture of carbon derived from ambient

Dissolved inorganic carbon (DIC) and that derived from diet (metabolic carbon). DIC has a distinct isotopic composition compared to metabolic carbon, and the proportions of each incorporated into otolith aragonite are controlled by the metabolism of the fish.

The ^{15}N in tissue is commonly used in ecological studies to determine trophic level, trophic structure and food chain length. This is possible, because the ratio of ^{15}N to ^{14}N increases as one moves from lower to higher levels of the food chain. Nitrogen isotopes are influenced by species, tissue, and type of consumer (e.g., carnivore, herbivore) and habitat type (marine, freshwater or terrestrial).

A part of above application the otolith play a vital role in many analysis/estimation like Stock discrimination, Presence of Diadromy, Presence of philopatry, Identification of juvenile nursery areas, As environmental proxies etc.

Conclusion

Otoliths are one of the most reliable tools for determining the age of a fish. Since age is used to establish growth rates of a fish species and age compositions of a certain population, otoliths are a powerful tool in fisheries management. Otolith chemistry and microstructure analysis have developed greatly in the recent years and have showed a wide range of applications for stock identification and other environmental studies concerning fish habitats. However, there are still several areas of otolith research that need further development. For example, to avoid overfishing, the management of deep sea fishes requires the development of new methods to validate the age determined from the otoliths, because many species appear to have long life spans and grow slowly. Also, the development of otolith growth models, apart from improving the accuracy of back–growth calculations, would simplify the methods for reconstructing life histories and environmental exposures. It is evident that otolith science will continue to make significant contributions to fundamental and applied research. Researchers continue to successfully extract highly relevant information from otoliths that is essentially inaccessible through other research approaches. Otolith science currently encompasses a broad range of research approaches that will likely continue to expand as new and improved technologies are developed.

Reference

Campana, S.E., Casselman, J.M.1993.Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci., 50:1062–1083

Campana, S.E., Chouinard, G.A., Hanson, J.M., Frechet, A., Brattey, J.2000.Otolith elemental fingerprints as biological tracers of fish stocks. Fish. Res., 46:343– 357

Campana, S.E., Neilson, J.D.1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci., 42:1014–1032

Campana, S.E.1999.Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser., 188:263–297

Chittaro, P.M., Fryer, B.J., Sale, P.F.2004. Discrimination of French grunts (Haemulon flavolineatum Desmarest, 1823) from mangrove and coral reef habitats using otolith microchemistry. J. Exp. Mar. Biol Ecol., 308:169–183

Ferreira, B.P., Russ. G.R. 1994.Age validation and estimation of growth rate of the coral trout, Plectropomus leopardus, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. Fish Bull., 92:46–57

Kalish, J.M.1990.Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and nonanadromous salmonids. Fish. Bull. (Wash DC), 88:657 - 666

Mulligan, T.J., Martin, F.D., Smucker, R.A., Wright, D.A.1987.A method of stock identification based on the elemental composition of striped bass Morone saxatilis (Walbaum) otoliths. J. Exp. Mar. Biol. Ecol, 114:241–248

Panfili, J., TomÁS, J., Morales-Nin, B. 2009. Otolith microstructure in tropical fish. In Tropical fish otoliths: information for assessment, management and ecology.Springer Netherlands.

Patterson, H.M., Swearer, S.E.2007.Long-distance dispersal and local retention of larvae as mechanisms of recruitment in an island population of a coral reef fish. Austra. Ecol., 32:122–130

Payan, P., Edeyer, A., de Pontual, H., Borelli, G., Boeuf, G., Mayer-Gostan, N. 1999. Chemical composition of saccular endolymph and otolith in fish inner ear: lack of spatial uniformity. Am. J. Physiol .,277:R123– R131

Radtke, R. L. 1985. Recruitment parameters resolved from structural and chemical components of juvenile Dascyllus albisella otoliths. Proc. 6th Int. Coral Reef Cong. 5:397–401.

Radtke, R. L. 1989. Strontium-calcium concentration ratios in fish otoliths as environmental indicators. Comp. Biochem. Physiol., 92: 198–194.

Smith, M.K.1992.Regional differences in otolith morphology of the deep slope red snapper Etelis carbunculus. Can. J. Fish. Aquat. Sci., 49:795–804

Smith, S.V., Buddemeier, R.W., Redalje, R.D., Houck, J.E.1979.Strontium-calcium thermometry in coral skeletons. Science, 204:404–407

Song, Z., He, C., Fu, Z., Shen, D.2008. Otolith thermal marking in larval Chinese sucker, Myxocyprinus asiaticus. Environ. Biol. Fish., 82:1–7

Swearer, S.E., Caselle, J.E., Lea, D.W., Warner, R.R.1999.Larval retention and recruitment in an island population of a coral-reef fish. Nature, 402:799–802

Thorrold, S.R., Jones, C.M., Campana, S.E.1997.Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker Micropogonias undulatus. Limnol. Oceanogr, 42:102–111

Thorrold, S.R., Latkoczy, C., Swart, P.K., Jones, C.M.2001.Natal homing in a marine fish metapopulation. Science, 291:297–299

Thorrold, S.R., Swearer, S.E.2009. Otolith chemistry. In Tropical fish otoliths: information for assessment, management and ecology .Springer Netherlands.

Thresher, R.E., Proctor, C.H., Gunn, J.S., Harrowfield, I.R. 1994. An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, Nemadactylus macropterus (Cheilodactylidae). Fish. Bull. (Wash DC), 92:817–840

Wilson, D.T., McCormick, M.I.1999.Microstructure of settlement-marks in the otoliths of tropical reef fishes. Mar. Biol., 134:29–41

Zhang, Z., Runham, N.W.1992.Temporal deposition of incremental and discontinuous zones in the otoliths of Oreochromis niloticus (L). J. Fish. Biol., 40:333–339.

Molecular taxonomy

Vikas Pathak, Shashi Bhushan, B. B. Nayak and Layana P.

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

DNA barcoding has also focused on the development of a global barcoding database as a species identification tool for large taxonomic assemblages of animals, representing a quick and easy method for non-specialists to identify different specimens. The identification process through DNA barcoding is relatively straight-forward, and depends upon the quantifiable matching of COI sequences from unknown specimens with previously documented and archived voucher specimens. The success of specimen identification depends on representation of barcodes of fishes from all over the world

DNA barcoding is a reliable taxonomic tool for confirmation of species (both plants & animals). Although the DNA sequences of related species are generally very similar, still differences in the sequence exist. The part of the DNA sequence is specific for species or for a particular organism and forms a unique and specific barcode of DNA.

DNA barcodes allow non-experts also to identify species, even from the damaged or industrially processed material. Short DNA barcodes, near about 700 nucleotides in length, can be quickly and unambiguously analyzed from thousands of specimens by computer programs (bioinformatic tools).

To meet these needs, a group of scientists joined forces in 2005 to launch the Fish Barcode of Life (FISH-BOL). The current FISH-BOL issue of Mitochondrial DNA provides a 5-year progress report on the campaign and updated –Collaborators' Protocol" to provide its continued growth and success. The implementation of standards is attributed to the success of barcoding program and at the end of this program, the new protocol aims to refine and further advance FISH-BOL best practices for the users.

In India, the pioneering work on fish taxonomy has been done by Hamilton (1822) under the name –An account of the fishes found in the river Ganges and its branches" which clarified ambiguity in identifying the fishes from regional names. After the Hamilton greatest initial contribution, the milestones in Indian ichthyology was –The Fauna of British India including Ceylon and Burma" by Day (1889).

Advances in Indian fish taxonomy took place during the twentieth century based on collections made from India and the neighboring countries. Most of these works pertain to freshwater fishes. Misra (1962) published "An aid to the identification of the common commercial fishes of India and Pakistan". Again in 1976, he published _The fauna of India and Adjacent countries (Pisces).

Methods employed in molecular Taxonomy

Hybridization

Genetic materials of two different genera are subjected to hybridize. Closely related genera exhibit a higher percentage of hybridization

DNA sequencing

DNA segments of different fish species are sequenced from one terminal to the other. The sequences of the different forms are the foundation of establishing similarity or dissimilarity among them.

Restriction mapping

Segments of DNA are extracted from different species of organism and subjected to restriction enzymes their after loaded to the agarose gel. Closely related genera have more similar bands on the resulted restriction map.

Chromosome banding

The chromosomes of different organisms are tested through a microscope and such banding of chromosomes are used for taxonomic purposes

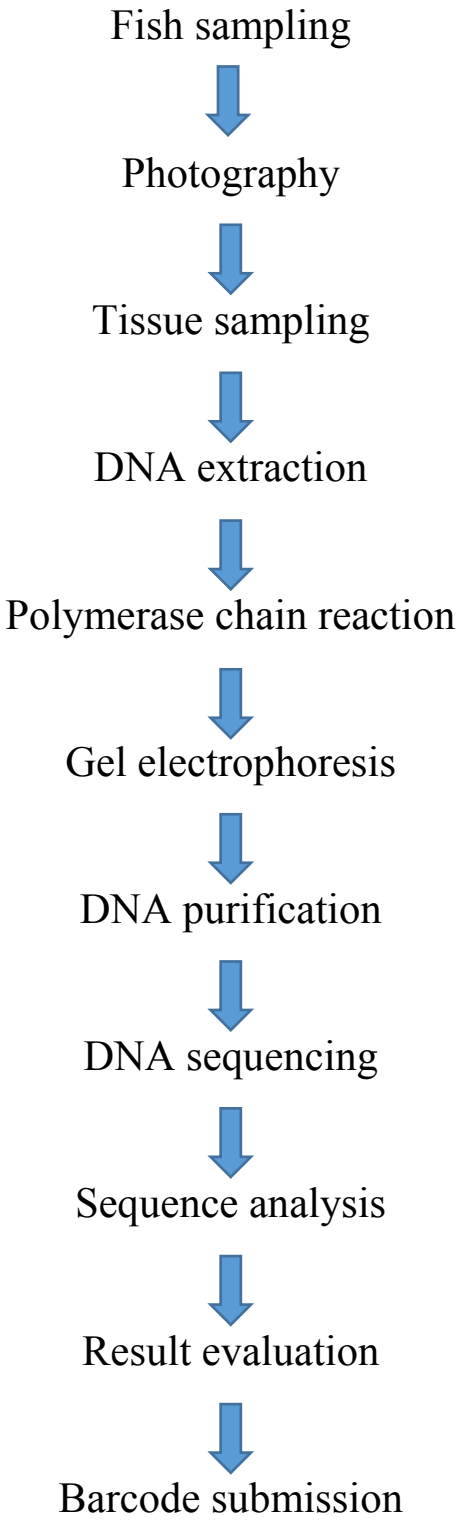
Amino acid sequencing

Similar to DNA sequencing, protein sequencing is also done. The amino acid sequence of a particular protein will be more similar among closely related genera.

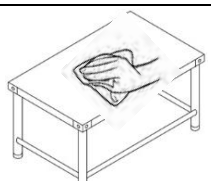
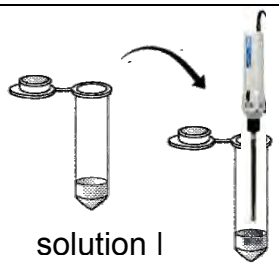
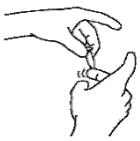
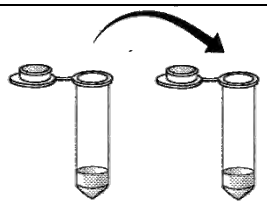
Immunological methods

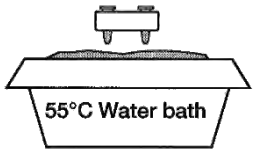
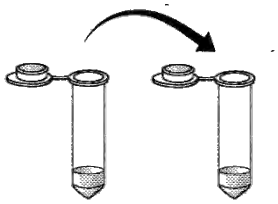
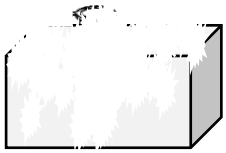
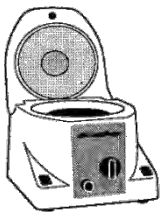
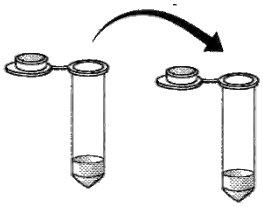
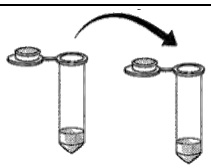
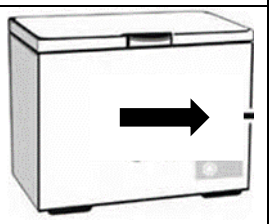
Antibodies which identify specific macromolecules/antigen, generally practice is done on the cell surface of the different organism. Antibodies can identify macromolecules from one species will often identify closely related species, but not from distantly related species.

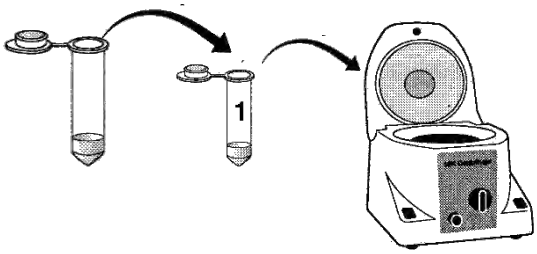
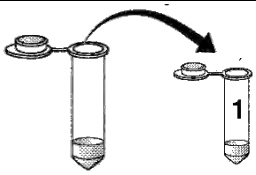
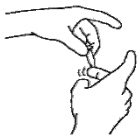
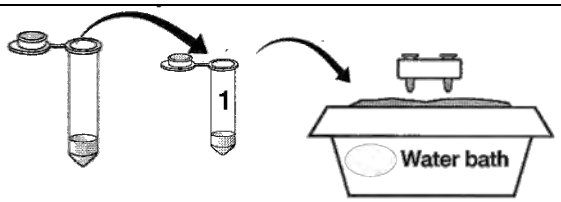
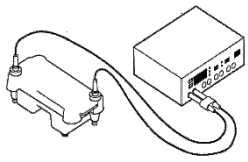
Flow chart depicting the steps involved in barcoding

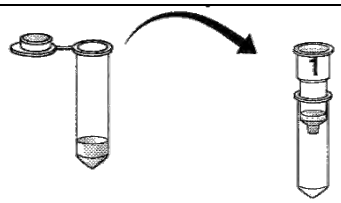
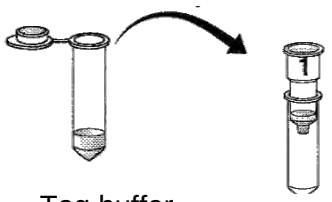
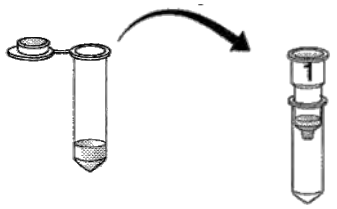
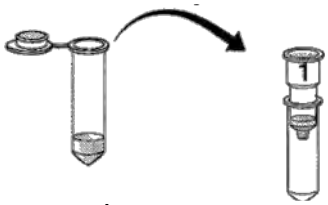
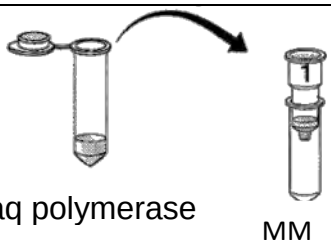
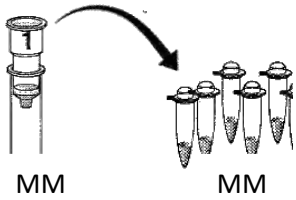


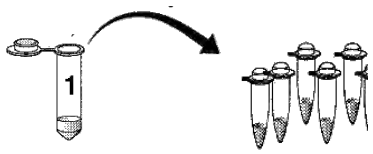
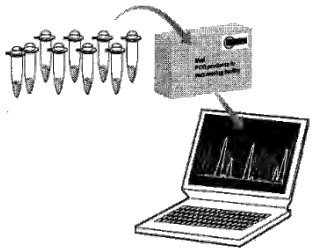
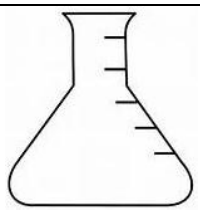
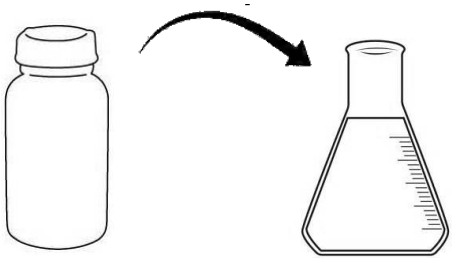
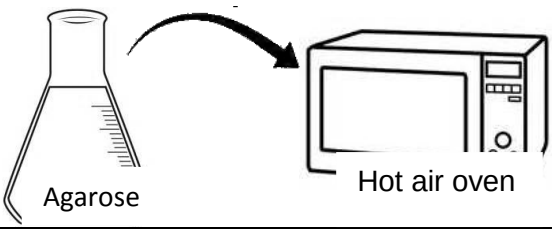
Quick guide of molecular taxonomy

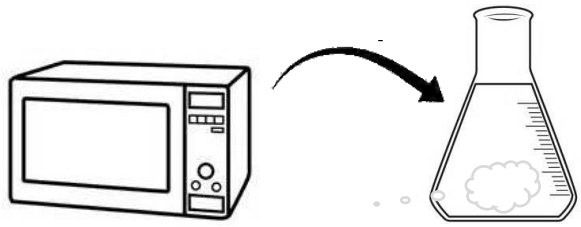
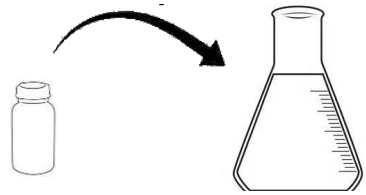
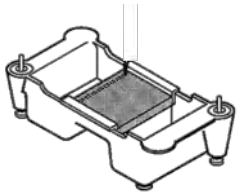
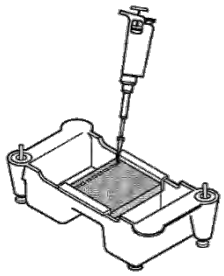
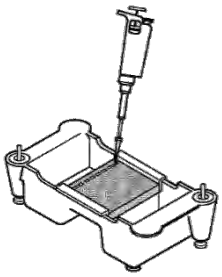
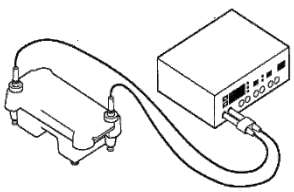
Quick guide for salting-out method	
Tissue sample collection	
Collect fish sample	 Fish sample
Remove tissue sample	 Tissue sample
Put sample tissue in ethanol filled vial	 Tissue in ethanol filled vial
DNA extraction	
First clean the working platform	 Cleaning the platform
Wash about 20mg of tissue in Millipore water and dry it in a tissue paper. Homogenize the tissue in 700µl solution I in 2 ml tube and flick the tube several time	 solution I Tissue homogenizing  Flick the tube
Add 10µl of proteinase K in the vial. Mix it properly and cover the lid with the help of paraffin film	 Add 10µl of proteinase K  Invert gently

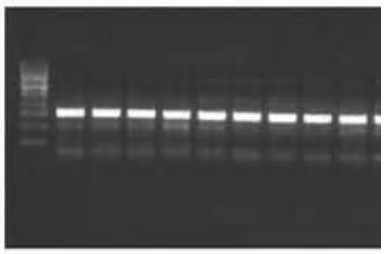
Incubate the mixture in a water bath of temperature 55° C overnight (18- 20 hrs)	 Water bath
Add 200 µl of solution Ⅱ (Table no. 5)and mix the aliquot by inverting the vial up and down	 Add 200 µl of solution Ⅱ  Inver gently
Keep it in ice (4 ° C) for 5 minutes	 Sample in ice box
Centrifuge the mixture at 8,000 rpm for 15 minutes 4° C	 Centrifugation for 5 minute
Take 1ml of transparent supernatant in 1.5 ml tube	 Take supernatant
Add 500 µl molecular grade absolute alcohol and keep it in -20° C for 1 hour	 Absolute alcohol 
Centrifuge at 10,000 rpm for 10 minutes at 4° C	

	 Centrifugation for 10 minute
Take the pellet and washed with 70% alcohol at 10,000 rpm for 10 minutes (Repeat the above procedure)	 Wash Centrifugation for 10 minute
Take the pellet and dried it under the fan of laminar flow	
Add 20 µl TE (10X) buffer and dissolve the pellet by flicking the tube	  TE buffer Flicking the tube
Add 1 µl RNase enzyme for removing the RNA and incubate one hour at 37° C (if necessary)	 RNase enzyme
Load it in 1% agarose gel for checking the DNA	  Gel loading Gel running
PCR (25µl reaction)	
Master-mix preparation	

Take 19.75 µl Nuclease free water	
Add 2.50 µl Taq buffer containing 1.5mM MgCl2	
Add 0.50 µl DNTPs	
Add 0.50 µl FI primer	
Add 0.50 µl RI primer	
Add 0.250 µl Taq polymerase	
Distribution of master-mix in PCR tubes	

Add 1 µl DNA template	 DNA template PCR tubes
Run the PCR for 35 cycle	 PCR
Gel electrophoresis	
Take Erlenmeyer flask of 50 ml	 Erlenmeyer flask
Add 50 ml of 0.5x (working buffer) TEN buffer (stock buffer 50x: To make working buffer take 10ml of stock buffer and add 990ml of NFDW)	 TEN buffer
Take 0.5gm of Agarose and add in TEN buffer	 Agarose TEN buffer
Keep it in oven for one minute to dissolve	 Agarose Hot air oven

After one minute remove from oven, let it dry till reaches 55 ^{0}C	 Hot air oven Agarose
After that add 2.5 µl of Ethidium bromide	 Ethidium bromide Agarose gel
Keep for 5 minute	
Pour it in comb fixed gel apparatus	
After semi solidifying transfer into cassette and Load the ladder in first well of gel	 Ladder loading
Load the DNA template along with dye	 DNA template loading
Provide the electric potential after connecting anode and cathode (60 – 80 volt) and run for 30 minutes	 Provide Electric potential

Check in UV light box	 DNA bands
DNA purification	Use purification kit and follow given protocol on purification kit
DNA sequencing	Follow standard protocol

Application of barcoding

Forensic applications

Food products

Most of the commercial species are available after the removal of some external morphological features as fresh (filleted, eviscerated, beheaded, skinned, etc.) and processed products (salted, marinated, smoked, frozen, canned, etc.), which makes a difficult task to species identification. For international fish marketing, large numbers of both processed fish products and exploited species explained why the substitution of a less valuable species for a valuable one (reflecting a commercial fraud), maybe a common activity difficult to detect.

For example, A study carried out on food fish in the United States and found that three-quarters of fish sold as the threatened ‗‗red snapper'' (*Lutjanus campechanus*) were mislabeled and belonged to other fish species. The application of these molecular methods helps not only to protect both producers and consumers from frauds but may also help to conserve fish species from illegal trafficking or over-exploitation.

Food poisoning

Identification of poisonous species and eliminate them from fish trade is one of the most important jobs of fishery inspection of food products. Differentiate the hazardous fishes (includes families of Molidae, Tetraodontidae, Canthigasteridae, and Diodontidae) from edible fishes or any fishery products which contain biotoxins, such as muscle paralyzing toxins or ciguatoxins. Hsieh and Hwang (2004) developed a PCR-RFLP assay to detect and identify 16 pufferfishes. This new assay, complete within 24 h and may be valuable to fishery officers to identify these poisonous fish.

Genetically modified organisms GMO detection

Consumer worries regarding genetically modified organisms (GMOs) have produced a demand for labeling fish foods derived from transgenic products. In prevision of mandatory or voluntary labeling, assays are being developed to identify GM fish food.

Masri et al. (2002) developed a PCR-specific primers protocol for the identification of genetically modified Coho salmon (Oncorhynchus kisutch). The primers were particular for the amplification of a gene develop present in transgenic salmon; no amplification from the wild-type fish. The PCR test could be completed in a few hours, by using tissues from all parts of fish including bones, blood, slime, scales and other internal organs.

Taxonomic applications

Species discrimination and identification

Traditionally, fish species identification is based on external morphological and anatomical features. Yet trust on external morphology can produce sometimes difficulties in cases where species may be very apparent differences or similar morphologically are misleading. Molecular genetic makers along with traditional taxonomic methods (integrative) of taxonomy can also help clarify relationships up to species level for example, integrative taxonomy help to resolve existence ambiguity among the sibling species (origin from the same parent but dissimilar in looking due to sexual dimorphism or different life stages) and even can be applicable on cryptic species (taxa that cannot be distinguished morphologically but are genetically distinct).

Identification of body parts

Large size shark species are difficult to identify as a whole and this task is more intimidating; if fish species are processed (entrails, fins removed and head). Within this context, Greig et al. (2005) produce a PCR-FINS method to identify shark species from the Atlantic fishery. They found that the sequence of the 12S–16S region of the mitochondrial DNA (mtDNA) contained sufficient information for discriminating shark species.

Ecological Applications

Young stages of fish are usually difficult to collect and identify. However, abundant numbers of fish species have distinctive larval phases; they vary from one stage to other in terms of both shape and size which is the important reason for the utilization of adults for identification. But this issue can be resolved by the application of molecular taxonomy.

Trophic relationships

Determining of trophic relationships within an ecosystem is a most important part of many ecological studies in that aquatic ecosystems need to be studied well alike terrestrial ecosystems. However, collecting authentic data on diet composition quantitatively and qualitatively for most species is fraught with difficulties. It is not always easy to get across

trophic interactions between prey and predators by direct observation, mainly when observing small animals with cryptic interlocked food chain ecology; but identification problem for tiny fishes and digested prey can be resolved by the application of DNA sequencing method.

Bibliography

Akasaki T, Yanagimoto T, Yamakami K, et al. 2006. Species identification and PCR RFLP analysis of cytochrome b gene in cod fish (Order Gadiformes) products. *J Food Sci* 71:190–195.

Amstrong, W. G. et al. 1985."Erratum: Fossil Proteins in Vertebrate Calcified Tissues". *Philosophical Transactions of the Royal Society B: BiologicalSciences* 309.1141 615-615.

Ardura, A., Pola, I.G., Ginuino, I., Gomes, V. and Garcia-Vazquez, E., 2010. Application of barcoding to Amazonian commercial fish labeling, *Food Research International,* 43(5): 1549-1552.

Armstrong, K. F., and Ball, S. L., 2005. DNA barcodes for biosecurity: invasive species Identification. *Phil. Trans. R. Soc. Land. B*., 360:1813-1823.

Babola O, Desvarenne S, Lacroix B, et al. 2004.L'identification des espe`cesanimal's dansl'alimentationhumaine et animale: un exemple d'application de la technologie des puces a` ADN. *Bull Soc Fr Micr* 19:30–36.

Berg, L. S., 1947. Classification of Fishes: both recent and fossils. Acad. Sci. U.S.S.R, 5(2): 86-517.

Bijl, Peter K., and HenkBrinkhuis.2015. 'A New Genus and Two New Species of Dinoflagellate Cysts from Lower Eocene Marine Sediments ofthe Wilkes Land Margin, Antarctica'. *Review of Palaeobotany and Palynology* 220,88-97.

Bloch, M. E. and Schneider, S. G., 1801. SystemaIchthyologiaeIconibus,valium 2 Berlin.

Bonants, P., Groenewald, E., Rasplus, J. Y., Maes, M., De Vos, P., Frey, J., Boonham, N., Nicolaisen, M., Bertacini, A., Robert, V., Barker, I., Kox, L., Ravnikar, M., Tomankova, K., Caffier, D., Li, M., Armstrong, K. F., Freitas-Astúa, J., Stefani, 34 E., Cubero, J. and Mostert, L., 2010. QBOL: a new EU project focusing on DNA barcoding of quarantine organisms. *EPPO Bulletin*, 40: 30–33.

Bucciarelli G, Golani D, Bernardi G.,2002. Genetic cryptic species as biological invaders: the case of a Lessepsian fish migrant, the hardyhead silverside Atherinomoruslacunosus. *J Exp Mar BiolEcol* 273:143–149.

Ce´spedes A, Garcı´a T, Carrera E, et al.. 1998. Identification of flatfish species using polymerase chain reaction (PCR) amplification and restriction analysis of the cytochrome b gene. *J Food Sci* 63:206–209.

Cuvier, G. and Valenciennes, A., 1836. Histoire Naturelles des Poissons. 22 volumes.

Darling, J., and Blum, M., 2007. DNA-based methods for monitoring invasive species: a review and prospectus. *Biological Invasions* 9: 751–765.

Galtier, N. and Gouy, M., 1995. Inferring phylogenies from DNA sequences of unequal base compositions, *Proceedings of the National Academy of Sciences of theUnited States of America*, 92(24): 11317.

Heist EJ, Gold JR., 1999. Genetic identification of sharks in the U.S. Atlantic large coastal shark fishery. *Fish Bull* (Wash D C) 97:53–61.

Hird HJ, Hold GL, Chisholm J, et al., 2005. Development of a method for the quantification of haddock (Melanogrammusaeglefinus) in commercial products using real-time PCR. *Eur Food Res Technol* 220:663–637.

Holmes, B. H., Steinke, D. and Ward, R.D., 2009. Identification of shark and ray fins using DNA barcoding. *Fish. Res*., 95: 280-288.

Hubert, N., Meyer, C.P., Bruggemann, H.J., Guérin, F., Komeno, R.J.L., Espiau, B., Causse, R., Williams, J.T. and Planes, S., 2012. Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-Barcoding provides new support to the Centre-of-overlap hypothesis, *PLoS One,* 7: 3.

Lakra ,W. S, Das, P and Sarkar, U. K., 2011, Fish Genetic Resources and Their conservation. In: S. Ayyappan, J. K. Jena, A. Gopalakrishnan and Dr. A. K. Pandey,ed., 2011. *Handbook of Fisheries and Aquaculture*. New Delhi, ICAR, 2:33-65.

Li Z, Nishimoto MM, Love MS, et al., 2006. Comparing the identification of southern California juvenile rockfish (genus Sebastes spp.) by restriction site analysis of the mitochondrial ND3/ND4 region and by morphological characteristics. *Fish Bull* (Wash D C) 104:376–382.

Lubbock, H.R. and Polunin, N., 1975. Conservation and the tropical marine aquarium trade, *Environmental Conservation,* 2(3): 229-232.

Malyutina, Marina V. 'New Data on Pacific Microcope (Crustacea, Isopoda, Munnopsidae) With Descriptions of Two New Species and Redescription of M. Ovata (Birstein, 1970)'. Deep Sea Research Part II: Topical Studies in Oceanography 111 (2014): 220-244.

Maretto F, Reffo E, Dalvit C, et al., 2007. Finding 16S rRNA gene-based SNPs for the genetic traceability of commercial species belonging to Gadiformes. *Ital J AnimSci* 6:161–163.

McDowell JR, Graves JE.,2002. Nuclear and mitochondrial DNA markers for specific identification of istiophorid and xiphiid billfishes. *Fish Bull* 100:537–544.

Michelini E, Cevenini L, Mezzanotte L *et. al.,* 2007. One-step triplex-polymerase chain reaction assay fot the authentication of yellowfin (Thunnusalbacares), bigeye (Thunnusobesus), and skipjack (Katsuwonuspelamis) tuna DNA from fresh, frozen, and canned tuna samples. J Agric Food Chem 55:7638–7647*Mol.Biol*. 42: 1-6.

Munro, I. S. R., 1955. The marine and freshwater fishes of Ceylon. Halstead press, Sydney. 103 225pp.

Murty, V. S., Kumaran, M. and Lalmohan, 1989. Resources of ornamental fishes. In: Marine Living Resources of the Union Territory of Lakshadweep, an indicative survey with suggestions for development, *Bulletin Central Marine FisheriesResearch Institute*, 43: 46-64.

Nazari, Vazrick, Evgueni V. Zakharov, and Felix A.H. Sperling. "Phylogeny, Historical

Nelso n, J.S., 1994. Fishes of the world. Third edition. New York: John Wiley and Sons,Pa¨a¨bo S, Poinar H, Serre D et al (2004) Genetic analyses from ancient DNA.

Annu Rev Genet 38:645–679 Paterlini M (2007) there shall be order. *The legacy of Linnaeus in the age of molecular biology*. EMBO Rep 8:814– 816.

Politov D, Gordon N, Afanasiev K, et al. 2000. Identification of palearcticcoregonid fish species using mtDNA and allozyme genetic markers. *J Fish Biol* 74:51–71.

Pyle, R. L., 1993. Marine aquarium fish.*In:* Near shore Marine Resources of the South Pacific. Information for fisheries development and management. (ed. Wright, A. and Hill, L.). Suva, Fiji: Institute of Pacific Studies, and Honiara, Solomon Islands: *Forum Fisheries Agency,* pp 135-176

Quinteiro J, Sotelo CG, Rehbein H *et al.* 1998. Use of mtDNA direct polymerase chain reaction (PCR) sequencing and PCR-restriction fragment length polymorphism methodologies in species identification of canned tuna. *J Agric Food Chem* 46:1662–1669.

Rehbein H, Mackie I, Pryde S.*et al.*, 1999. Fish species identification in canned tuna by PCR-SSCP: validation by a collaborative study and investigation of intra-species variability of the DNA-patterns. *Food Chem* 64:263–268.

Rubinoff, D., 2006. DNA barcoding evolves into the familiar. *Conservation Biology* 20(5): 1548-1549.

Rubinoff, D., Cameron, S., and Will, K., 2006. A genomic perspective on the Shortcomings of mitochondrial DNA for –barcoding" identification.*Journal of Heredity,* 97: 581594.

Some blood parameters in sea bass, Dicentrarchuslabrax, infected by bacteria, virus and parasites. *J. Fish Biol*. 31(Suppl A):259- 261.

Steinke, D., Zemlak, T.S. and Hebert, P.D.N., 2009. Barcoding Nemo: DNA-Based Identifications for the Ornamental Fish Trade. *PLoS One* (4): 7.

Strange R, Stepien C., 2007. Yellow (Percaflavescens) and Eurasian (P. fluviatilis) perch distinguished in fried fish samples by DNA analysis. Fish Bull (Wash D C) 105:292–295.

Tamura, K. and Nei, M.,1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *MolecularBiology and Evolution,* 10: 512-526.

Wabnitz, C., Taylor, M., Green, E. and Razak, T., 2003. From ocean to aquarium. United Nations environment programme-world conservation monitoring centre,Cambridge, UK.

Ward, R.D., Holmes, B.H., White, W.T. and Last, P., 2008. DNA barcoding Australian Chondrichthyans: results and potential uses in conservation. *Marinefreshwater resource.* 59: 57-71.

Will KW, Rubinoff D., 2004. Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics* 20:47–55.

Will, Kipling W. and Daniel Rubinoff, 2004. "Myth Of The Molecule: DNA Barcodes For Species Cannot Replace Morphology For Identification And Classification". *Cladistics* 20.1: 47-55.

Winfield, Ignacio, Manuel Ortiz, and Sergio Cházaro- Olvera, 2009. 'A New Sponge-Inhabiting Amphipod Species (Crustacea, Gammaridea, Sebidae) From the Veracruz Coral Reef System, Southwestern Gulf Of Mexico'. *Organisms Diversity & Evolution* 9.1:72.e1-72.e8.

Yang D, Cannon A, Saunders S., 2004. DNA species identification of archaeological salmon bone from the Pacific Northwest Coast of North America. *J ArchaeolSci* 31:619–631.

Zhang,D.X. and Hewitt, G.M., 1996. Nuclear integration: challenge for mitochondrial DNA markers. *Trends Ecol. Evol*. 11: 247-251.(doi:10.1016/ 0169 - 5347(96)10031-8.).

Zhang J, Huang L, Huo H., 2004. Larval identification of Lutjanus Bloch in Nansha coral reefs by AFLP molecular method. *J Exp Mar BiolEcol* 298:3–20.

Genetic population structure and its applications in fisheries management

Sri Hari M, Shashi Bhushan and Vikas Pathak

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Stock Concept

Stocks are the primary interest in fisheries management. The stock concept in fisheries is gaining importance regarding wise use of resources for food and preserving the genetic diversity (Booke, 1999). The stock concept contains two basic principles: that fish are subdivided into local populations, and that there are genetic differences between local populations which are adaptive recently, definition for stock is given as ~~–a~~ part of a fish population usually with a particular migration pattern, specific spawning grounds, and subject to a distinct fishery" (ICES, 2012). Stocks with a low level of integrity may be termed 'harvest stocks', which are defined as "locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource" (Gauldie, 1988) In contrast to most stock definitions, this concept does not imply any genetic or phenotypic differences between stocks.

Phenotypic stock is a group of fish that has the characteristics which are expressed in one or more ways depending on the type of environment while Genetic stock is a reproductively isolated from population of species. The phenotypic and harvest stock definitions are entirely different in fisheries management perspective. The short-term fisheries management concerns only about economic advantages, for the long-term fisheries management for the support of molecular genetic research is essential. Lack of knowledge on the exact number of interbreeding populations of an exploited species will not help the management policies to achieve long-term conservation goals.

(Stock and Population terms are now used interchangeably. The term "stock" is generally used by a fishery biologist whereas the term "population" used by a fish geneticist.)

Genetic Population Structure

Population can be defined as a group of conspecific individuals forming a breeding unit sharing a particular habitat at a certain time (Slatkin, 1993). Population genetics is concerned with the analysis of demographic and evolutionary factors affecting the genetic composition of a population. Genetic differences in neutral loci are usually considered as the

–true" indicators of stock structure. The main subject of population genetics is to study the genetic variation in the population and its change, the following of allele frequencies through time and space and because of natal homing, the spatial isolation protects the gene flow across the population. The pattern for distribution of genetic variation within and between populations is referred to as the genetic population structure of the species. In the short term, three processes are potentially important in influencing genetic variability over space: genetic drift, selection, and gene flow (Cadrin et al., 2004). Gene flow among subpopulations is a characteristic attribute of population genetic studies. Measuring genetic diversity in wild fish populations or aquaculture stocks is essential for interpretation, understanding and effective management of these populations or stocks. Gene flow will not always happen when a population moves across the areas because of the temporal and spatial variation between the stocks. Most of the marine species usually show low genetic differentiation among populations. Loss of genetic diversity within populations might be associated with inbreeding depression, which in turn results in reduced fitness and ultimately jeopardizes the population persistence.

The development of DNA based markers revolutionized the had revolutionized the area of population genetics. Molecular marker is a DNA sequence which is used to track a particular locus on a marker gene. The advantages of molecular markers include, they provide direct insights into the population structure, used for selective breeding programmes and can be used transgenerationally i.e., to examine the interbreeding of hatchery and wild populations. There are three basic types of molecular markers that are most commonly used in population structure of fishes: 1) Allozyme 2) DNA Markers. Allozymes are the first-generation molecular markers which gain widespread use in delineating the populations of fishes. Use of allozymes as markers for population studies, needs a fresh sample as the allozymes degrades rapidly after death at higher temperatures and the use of allozyme is not advisable where immediate freezing of tissue sample is not possible. Further, DNA markers can be classified into Mitochondrial and Nuclear markers based on transmission and evolutionary dynamics. Even though, there are several markers available, no markers are superior over other and depending upon the available resources, time and problem need to be addressed, some markers can be addressed to a certain problem.

Mitochondrial markers

Molecular markers can be categorized into two viz., nuclear DNA and mitochondrial DNA (mtDNA) markers based on their transmission and evolutionary dynamics (Park and Moran, 1994). Mitochondrial DNA (mtDNA) is a small, double-stranded circular DNA molecule contained in multiple copies in the mitochondria. Interms of gene content, animal

mitochondrial genome is stable and it contains 13 gene coding proteins, 22 genes for transfer RNA, 2 genes for ribosomal RNA and 1 non – coding region (D-loop). mtDNA has a highly mutation rate and characterized my high level of polymorphism and divergence and useful in differentiating the closely related population. As they consider fixed genotypic, instead of considering environmentally induced phenotypes. Offshore fishes show very little genetic differentiation than inshore pelagic fishes which shows a high level of population differentiation. The major features of mtDNA: a) in general maternally inherited a haploid single molecule; b) the entire genome is transcribed as a unit; c) not subject to recombination and provides homologous markers; d) mainly selectively neutral and occurs in multiple copies in each cell; e) replication is continuous, unidirectional and symmetrical without any apparent editing or repair mechanism; and f) optimal size, with no introns present.

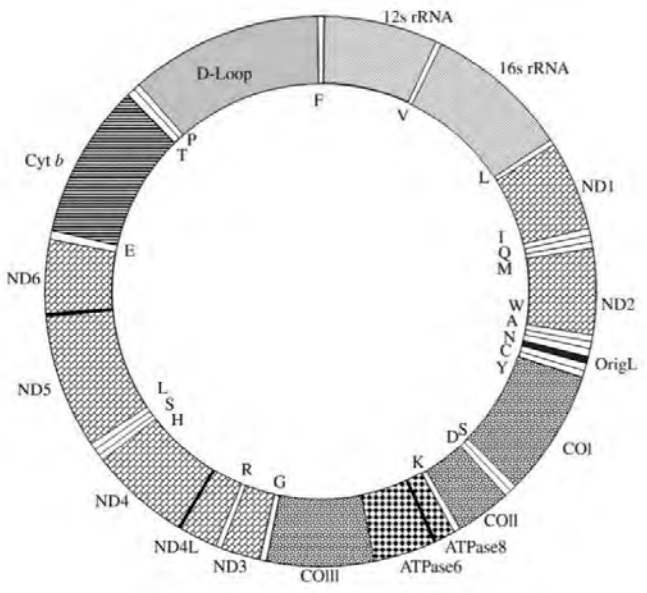


Figure 1: Vertebrate Mitochondrial Genome (Brown, 2008).

Mitochondrial DNA evolves 5-10 times as rapidly as Nuclear DNA (Brown *et al.,* 1979), thus providing the researchers the small differences between the populations and closely related species. Like nuclear DNA the mitochondrial genome includes coding and non-coding regions and later evolves much faster than coding regions of DNA (Avise, 1994). The technical advantages, such as the requirement of only small amounts of fresh, frozen or alcohol-stored tissue, to amplify the genes has also made mtDNA a very practical genetic tool.

Population studies by using mtDNA require small sample size than allozyme markers (Carvalho and Hauser, 1994). Since mtDNA is haploid, non-recombining and inherited maternally, the effective population size is small for mtDNA when compared to nuclear

DNA. Numerous phylogenetic studies have relied on the mtDNA genes amplification through conserved polymerase chain reaction (PCR) primers. Though microsatellite markers help in analysing the population structure at finer scales, they are less informative at larger scales of divergence (Shaw *et al.*, 1999). Hence, it acts as a more sensitive indicator of population phenomena such as bottlenecks and hybridization.

Cytochrome *b* (Cyt *b*) gene is used to study species specific pattern in several fishes. Primarily because (1) evolutionary dynamics of the Cyt *b* gene and the biochemistry of the proteins produced are better characterized than most other molecular systems and levels of genetic divergence typically associated with sister sps and co-familiar genera usually are in a range in which the Cyt *b* gene is phylogenetically informative and unlikely to be severely compromised by saturation effects involving superimposed nucleotide substitution.

ATPase 6 and 8 are the two genes with overlapping sequences in between, codes for ATPase enzyme that provides energy for the cell to use through the synthesis of adenosine triphosphate (ATP). ATPase gene of mtDNA is comparatively faster evolving and is extremely useful in assessing population structure, levels of connectivity and influence of historical processes in fish species.

D-loop region also called as the mitochondrial DNA control region in vertebrates, does not code for a functional gene and therefore leads to a high average substitution rate. It is usually the fastest evolving region in the mitochondrial DNA of vertebrates and invertebrates and therefore more sensitive than protein loci as a marker to score intraspecific variations of many organisms.

Use of genetic structure in fisheries management

Genetics and Fishery management can interact in both ways. Genetic population structure of a species is used to find the distribution of subpopulations and the harvest of the subpopulations can be regulated to protect the weaker populations based on the distributions. Effective conservation often depends on the identification of management units and timely information regarding the effects of natural and anthropogenic factors on movement and gene flow between these units, temporal sampling can provide richer insights into the gene flow of the populations.

Nowadays artificial propagation has been increasingly used for consecration and management of fishery resources and releasing of captively produced individuals into the wild might cause serious effects on the wild populations. Hence, it is necessary to monitor the

genetic changes in the captive populations and molecular markers can also be used in parentage analysis to assess reproductive success of captive individuals in the wild.

Restocking and stock enhancement are the important options of fisheries management programme, swamping of the natural population with the group which has different genetic make-up from outside or escape of hatchery reared fishes into the wild leads to extinction, loss of population identity, loss of between population genetic variation, inbreeding depression, loss of disease response etc which relies on genotypic diversity. To overcome these possible negative effects, the population structure of the species needs to be studied before involved in restocking and stock enhancement programmes. Although phenotypic methods do not provide evidence for separate breeding populations, they can be more appropriate tools for defining stocks than genetic studies. This is because the small amount of interchange between population~ which is necessary to maintain genetic homogeneity might be unimportant by fishery-management standards.

Due to the wide variety of approaches, rapidly advancing technologies, difficulties in sampling and also the conflicting terminologies makes stock/population identification the one of the most perplexing research areas in fisheries science, a single methodological approach to identify the management unit of the species is not reliable. Identification of fish stocks and their genetic variability is considered to be important for scientific and judicial exploitation of the resources. Since, stock identification is a constantly evolving and multidisciplinary filed encompassing many techniques, combining the results obtained with several methods might provide possible insights into the stock structure of the species.

Reference

Booke, H.E., 1981. The conundrum of the stock concept—are nature and nurture definable in fishery science?. *Canadian Journal of Fisheries and Aquatic Sciences*, 38(12):1479-1480.

ICES, 2012. International Council for the Exploration of the Sea Advice 2012, Book 1. pp.4

Gauldie, R.W., 1988. Tagging and genetically isolated stocks of fish: a test of one stock hypothesis and the development of another. *Journal of applied ichthyology*, 4(4):168-173.

Cadrin, S.X., K.D. Friedland, and J.R. Waldman, eds. 2004. Stock Identification Methods: Applications in Fishery Science. Elsevier Press,736pp

Park, L.K. and Moran, P., 1994. Developments in molecular genetic techniques in fisheries. *Reviews in Fish Biology and Fisheries*, 4(3):272-299.

Brown, W.M., George, M. and Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences, 76(4):1967-1971.

Carvalho, G.R. and Hauser, L., 1995. Molecular genetics and the stock concept in fisheries. In *Molecular genetics in fisheries* (pp. 55-79). Springer, Dordrecht.

Shaw, P.W., Pierce, G.J. and Boyle, P.R., 1999. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. *Molecular Ecology*, *8*(3), pp.407-417.

Carvalho, G.R. and Hauser, L., 1995. Molecular genetics and the stock concept in fisheries. In Molecular genetics in fisheries (pp. 55-79). Springer, Dordrecht.

Çiftci, Y. and Okumuş, İ., 2002. Fish population genetics and applications of molecular markers to fisheries and aquaculture: I-Basic principles of fish population genetics. *Turkish Journal of Fisheries and Aquatic Sciences*, *2*(2), pp.145-155.

Primmer, C., 2006. Genetic characterization of populations and its use in conservation decision-making in fish. *The Role of Biotechnology in Exploring and Protecting Agricultural Genetic Resources. FAO, Rome*, pp.97-104.

Otolith SHAPE Analysis

Vikas Pathak, Karan K. Ramteke, Layana P. and Shashi Bhushan

ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

How to install "SHAPE"

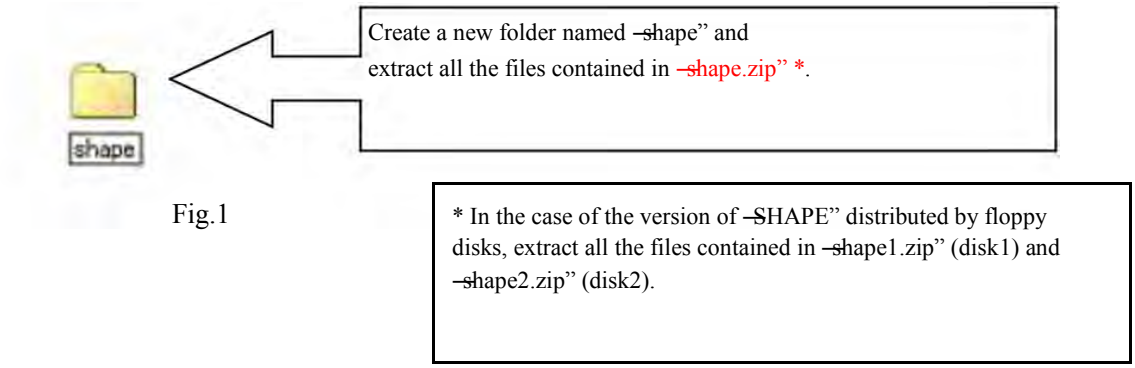


Fig.1

- Create a new folder named –shape" in your computer (Fig. 1).
- Extract all the files contained in —lsape.zip" to the —lsape" folder.

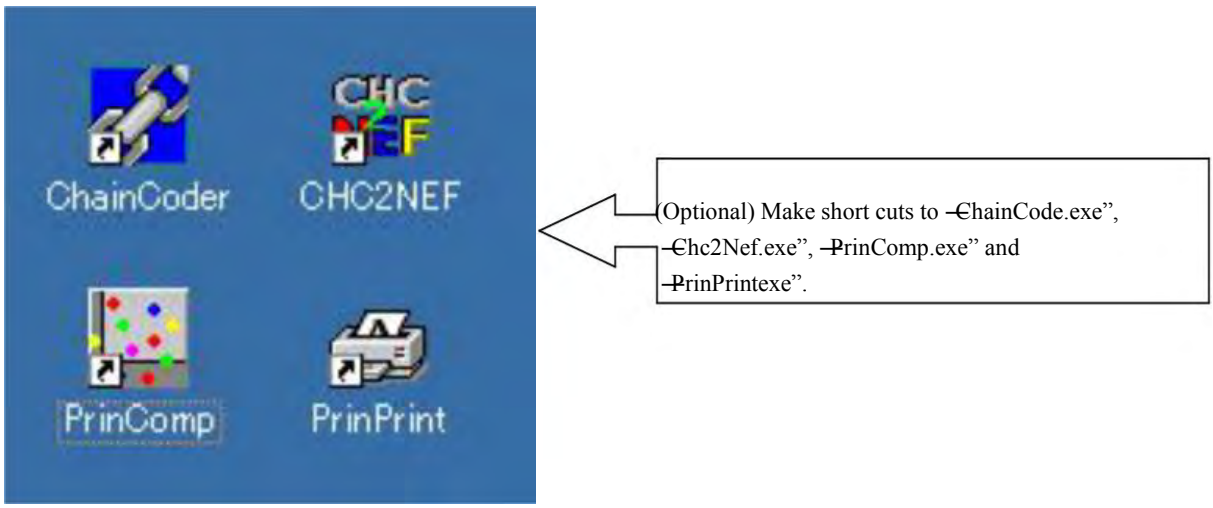


Fig. 2

3. (Optional) For convenience, make short cuts to "ChainCode.exe", "Chc2Nef.exe", "PrinComp.exe" and "PrinPrint.exe" on your desktop (Fig. 2).

How to try out "SHAPE" with sample files

This session gives instructions on how to try out —SHAPE" with the sample files contained in the package. First, please try out —ChainCoder" with the sample file named —Sample_img.bmp" which contains the image of five radish roots. —ChainCoder" extracts the contours of objects and stores the relevant information as chain-code. Next, please try out —Chc2Nef" with the sample file named —Sample_chc.chc" which contains chain-code data of c.a. 150 radish roots, or with the output file which you will make as a result of trying out —ChainCoder". –Chc2Nef" provides the normalized elliptic Fourier descriptors (NEFDs) from chain-coded contours. Finaly, please try out —PrinComp" and –PrinPrint" with the sample file named —Sample_nef.nef" which contains sufficient number of NEFDs data (276 radish roots) for principal component analysis. —PrinComp" performs the principal component analysis of the coefficients of the EFD, and –PrinPrint" visualizes the shape variation accounted for by each principal component. I hope that you become skilled in using —SHAPE" for your own samples through the practice of this tutorial.

First Step: Try out "ChainCoder"

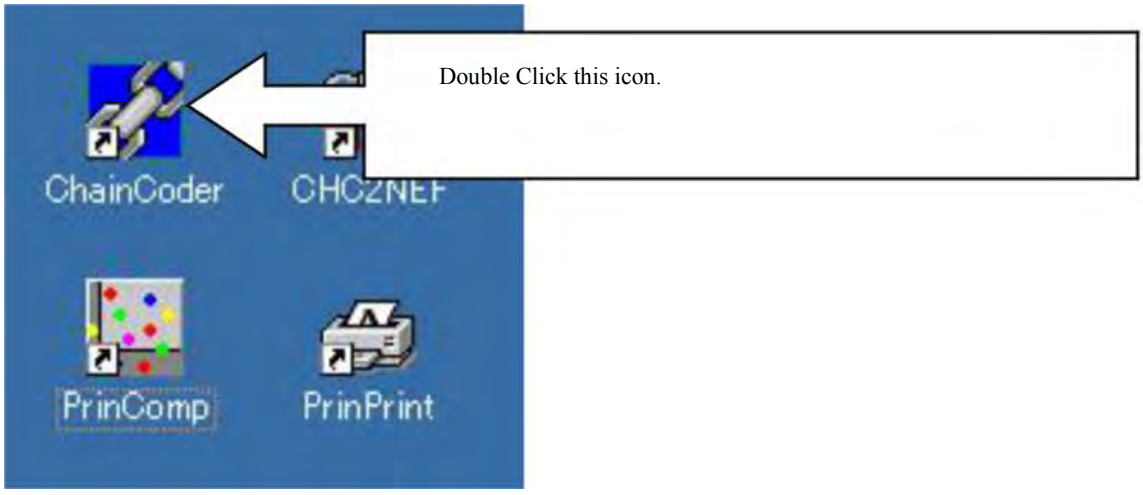


Fig.3

1. Start ChainCoder with double-clicking the —ChainCoder" icon (Fig. 3)

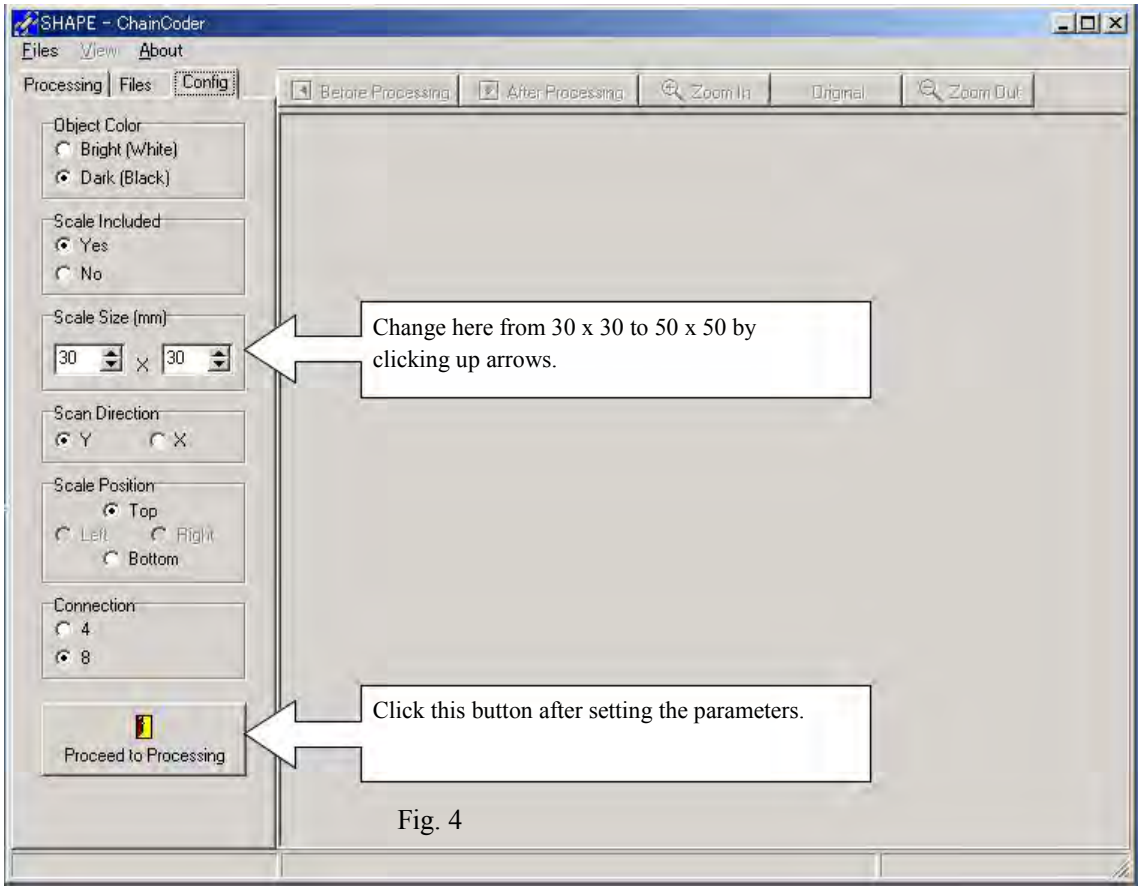


Fig. 4

2. Set or confirm the analysis parameters as follows (A-E) (Fig. 4).
   A) Object Color -> Dark (Default)
   B) Scale Included -> Yes (Default)
   C) Scale Size -> 50 x 50 (mm) (Click up arrows)
   D) Scan Direction -> Y (Default)
   E) Scale position -> Top (Default)

3. Click the –Proceed to Processing" button (Fig. 4).

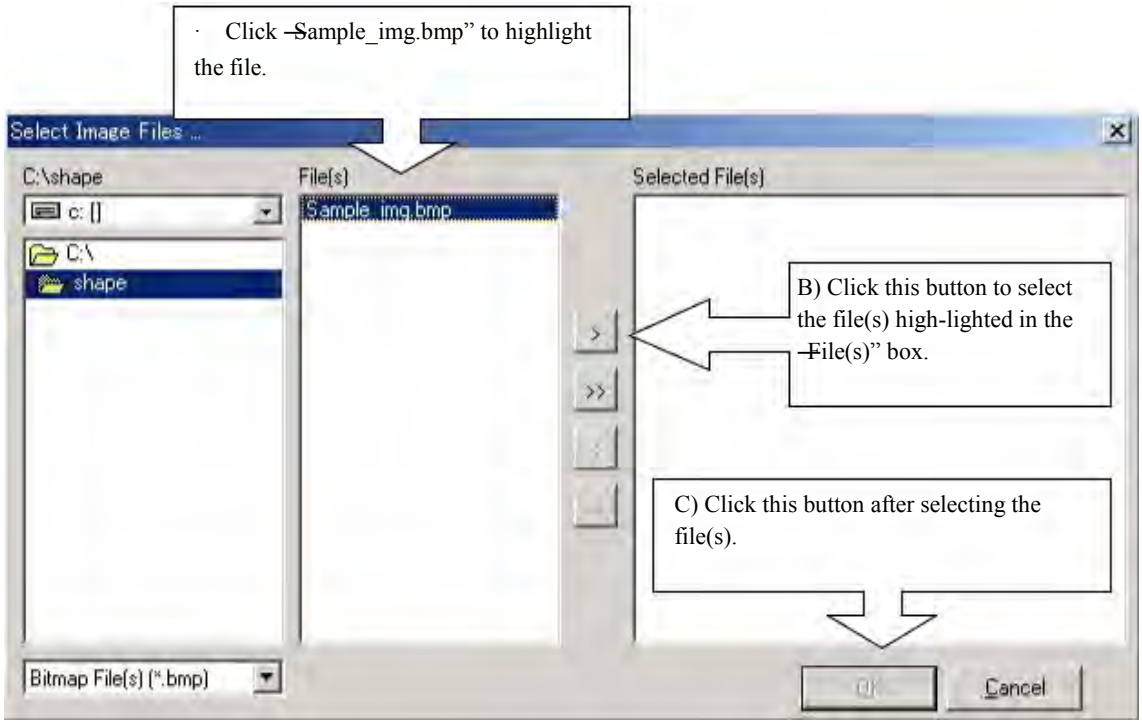


Fig. 5

· Select the image file(s) that you are going to analyze as follows (A-C) (Fig. 5).

Click the —Sample_img.bmp" contained in the shape folder.

Click the [>] button, and —Sample_img.bmp" will appear in the "Selected File(s)" box.

Click the –OK" button.

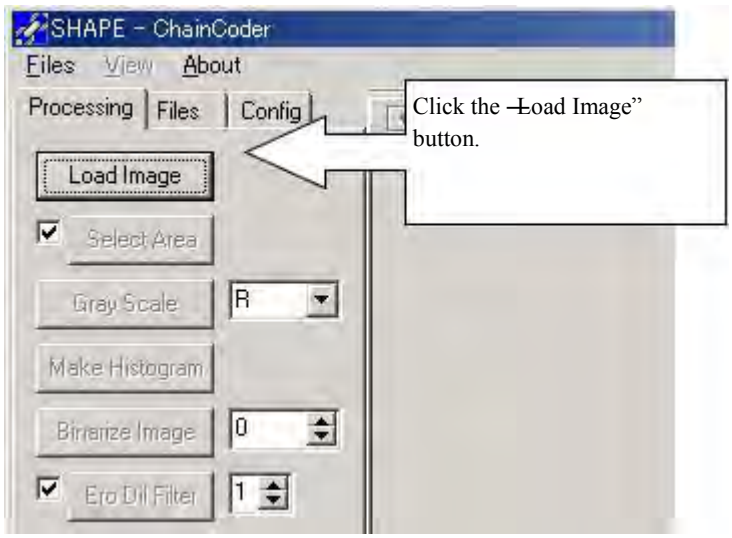


Fig. 6

5. Click the —Load Image" button to load the image into the program (Fig. 6).

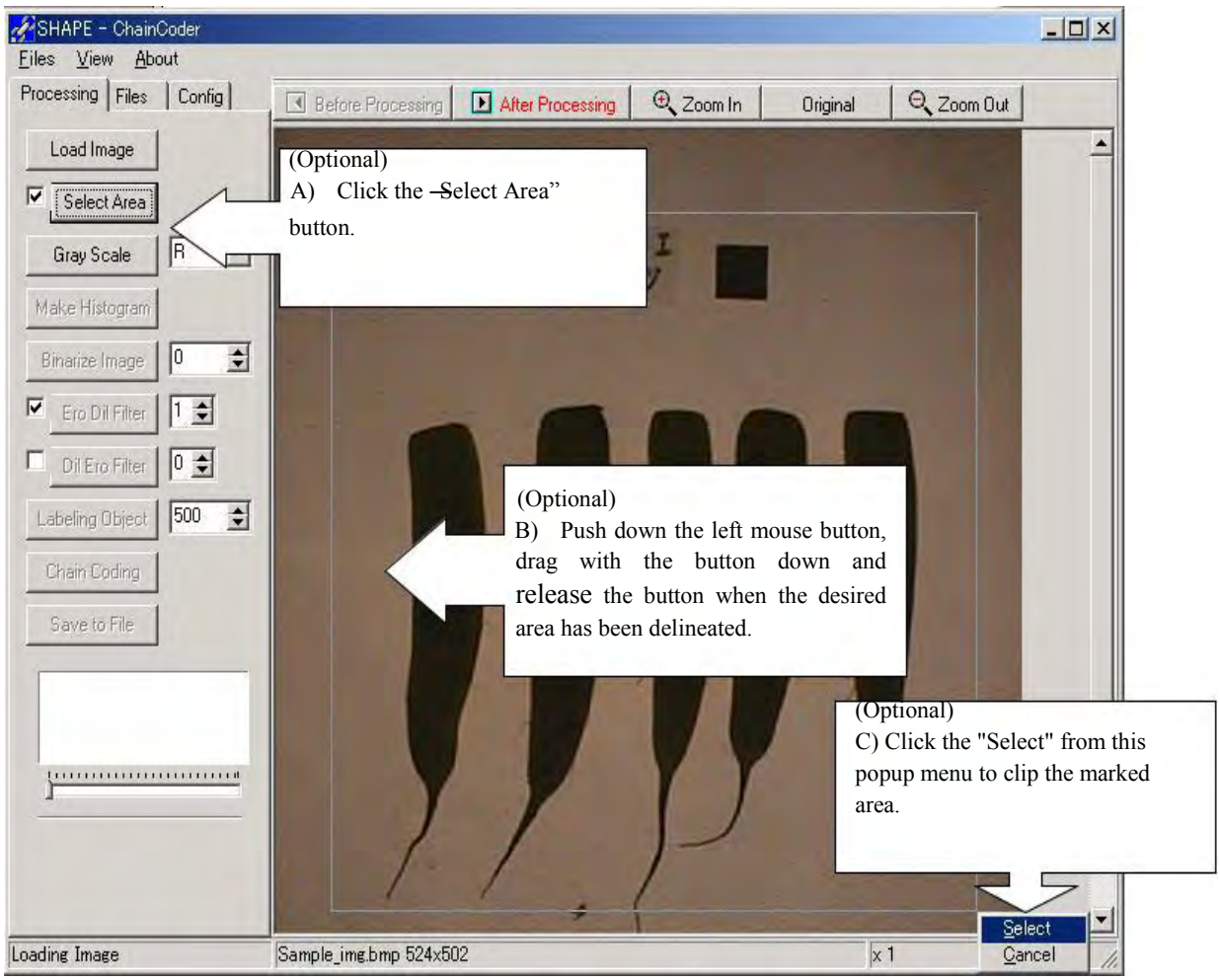


Fig. 7

6. (Optional) If you want to process the part of the image, select the processing area as follows (A-C) (Fig. 7)
   A) Click the —Select Area" button, and the mouse cursor will change shape from an arrow to a cross in the image window.
   B) Push down the left mouse button, drag with the button down and release the button when the desired area has been delineated.
   C) Click the "Select" from this popup menu to clip the marked area.

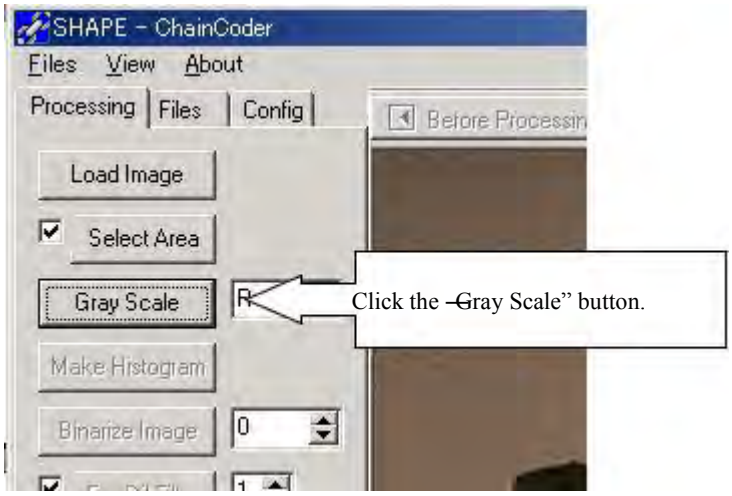


Fig. 8

7. Click the –Gray Scale" button to change a full color bitmap image to gray-scale.

! **Attention:** *This program can only handle FULL COLOR (24 bits) BITMAP images, and cannot handle directly 256 COLOR, 16 COLOR and MONOCHROME BITMAP images or JPEG images. So, if you have files of images in a different format, such as jpeg or less colored bitmap (black and white, 16 colors or 256 colors bitmap), you have to convert them to full color (24 bits) bitmap format prior to analysis using the graphic programs such as "Microsoft Paint" that comes with Microsoft Windows.*

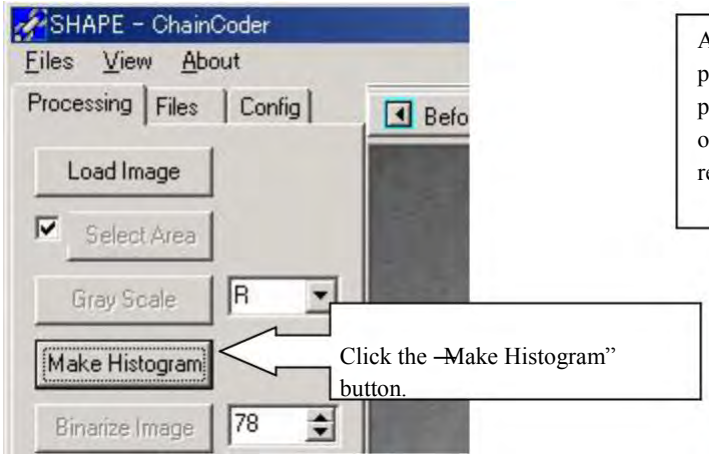


Fig. 9

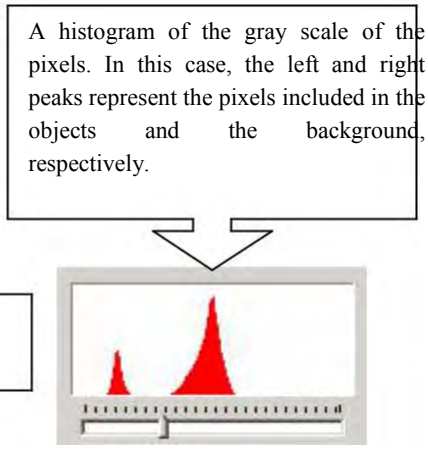


Fig. 10

8. Click the —Make Histogram" button (Fig. 9), and a histogram of the gray scale of the pixels will then be shown in the histogram box (Fig. 10). Through this step, an appropriate threshold value is determined and appeared in the box beside the –Binarize Image" button.

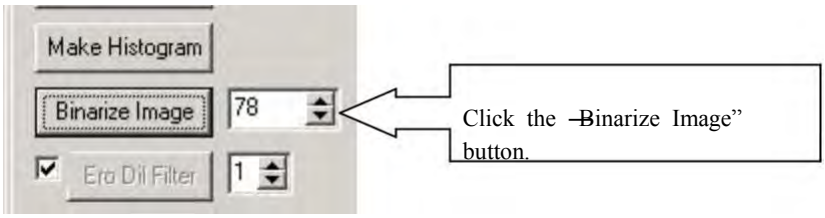


Fig. 11

9. Click the "Binarize Image" button, to convert the gray scale image to a binary image in which the objects and background are represented as 1 (white) and 0 (black), respectively (Fig. 11). If you want to adjust threshold value for the binarization, change the value in the box beside the "Binarize Image" button by clicking the up or down buttons, as appropriate (Fig. 11). The value can be changed by dragging the pointer on the ruler bar below the histogram box (Fig. 10).

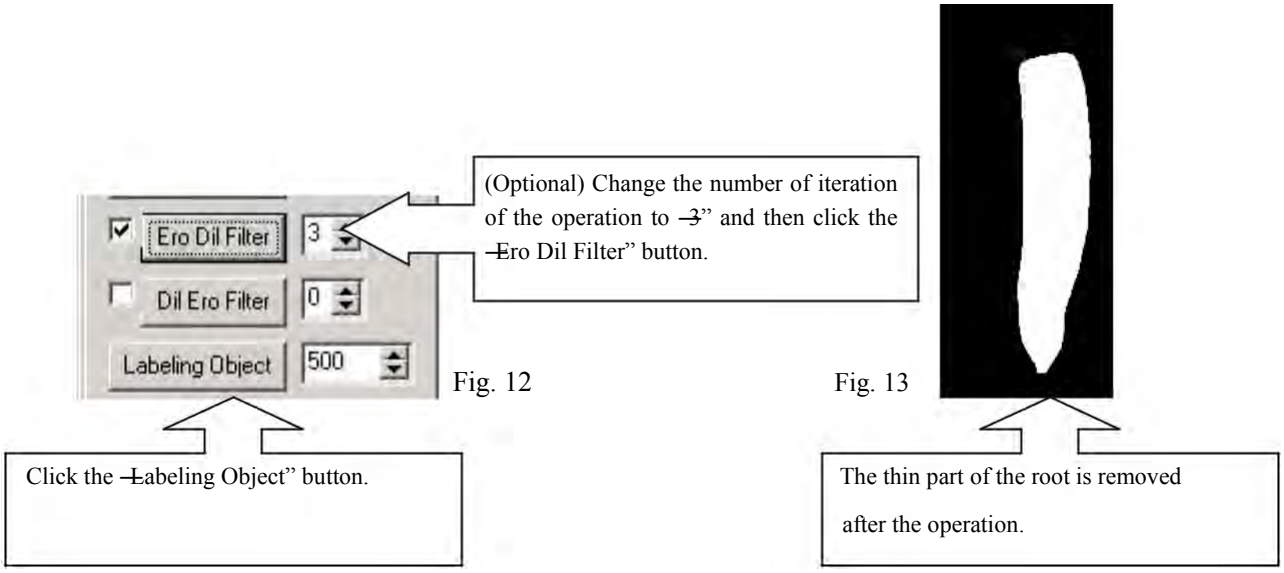
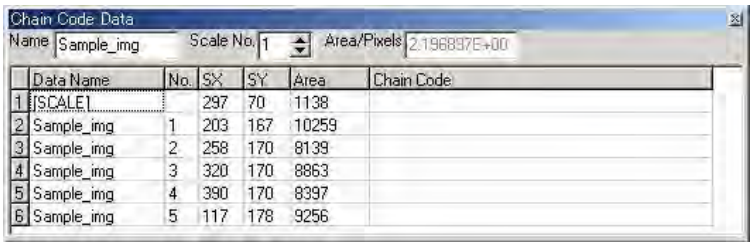


Fig. 12

Fig. 13

10. (Optional) Change the number of iteration of the operation from "1" to "3" and click the —Ero Dil Filter" button (Fig. 12). Then, the noises and the thin part of the roots will be removed (Fig. 13).



	Data Name	No.	SX	SY	Area	Chain Code
1	[SCALE]		297	70	1138	
2	Sample_img	1	203	167	10259	
3	Sample_img	2	258	170	8139	
4	Sample_img	3	320	170	8863	
5	Sample_img	4	390	170	8397	
6	Sample_img	5	117	178	9256	

Fig. 14

11. Click the —Labeling Object" button (Fig. 12), and each object will then be numbered and displayed in the —Chain Code Data" window (Fig. 14).

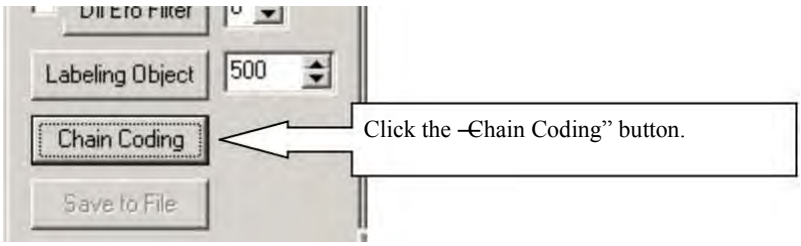


Fig. 15

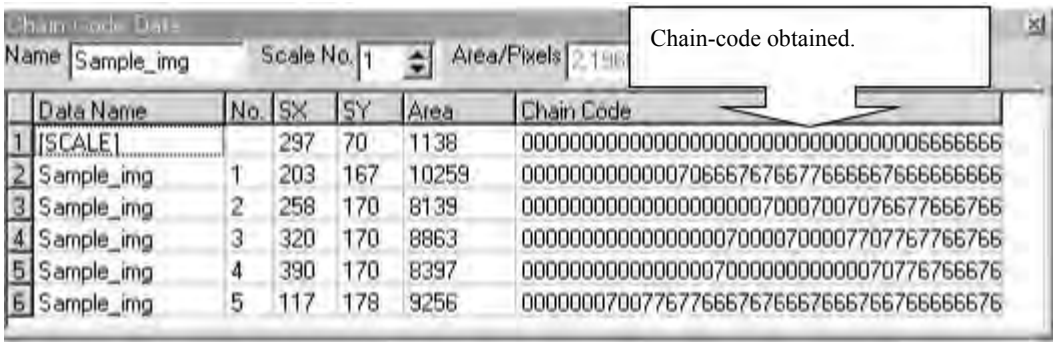


Fig. 16

12. Click the "Chain Coding" button, to obtain the chain-code for each object (Fig. 15). The chain-code obtained will be displayed in the —Chai Code Data" window (Fig. 16).

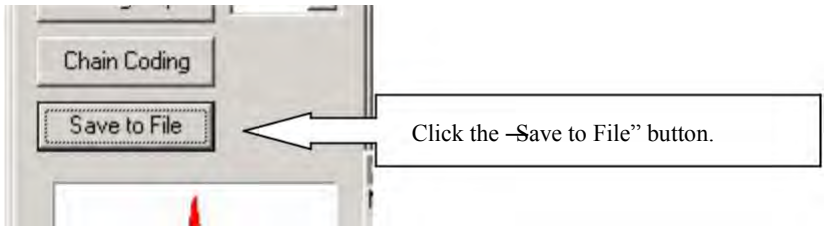


Fig. 17

13. Click the "Save to File" button, the chain code data will then be saved in an output file. In the first time the button is used after executing this program, the "Save Chc File dialog" window will appear. Input the name of the output file as, for example, —est.chc" and save the data. *After saving the data for the first image, you can also process remaining multiple files with repeating the steps 5 to 13. Detail information for processing multiple files is described in the manual of SHAPE v.1.3.*

14. Close –Chain Coder".

Second Step: Try out "Chc2Nef"

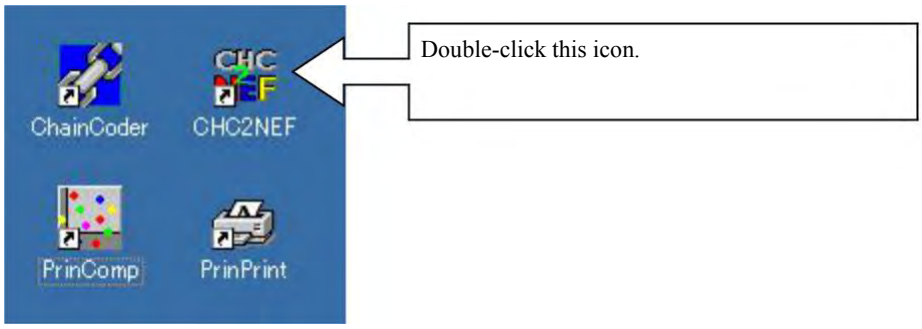


Fig. 18

15. Start —Chc2Nef" with double clicking the —Chc2Nef" icon (Fig. 18).

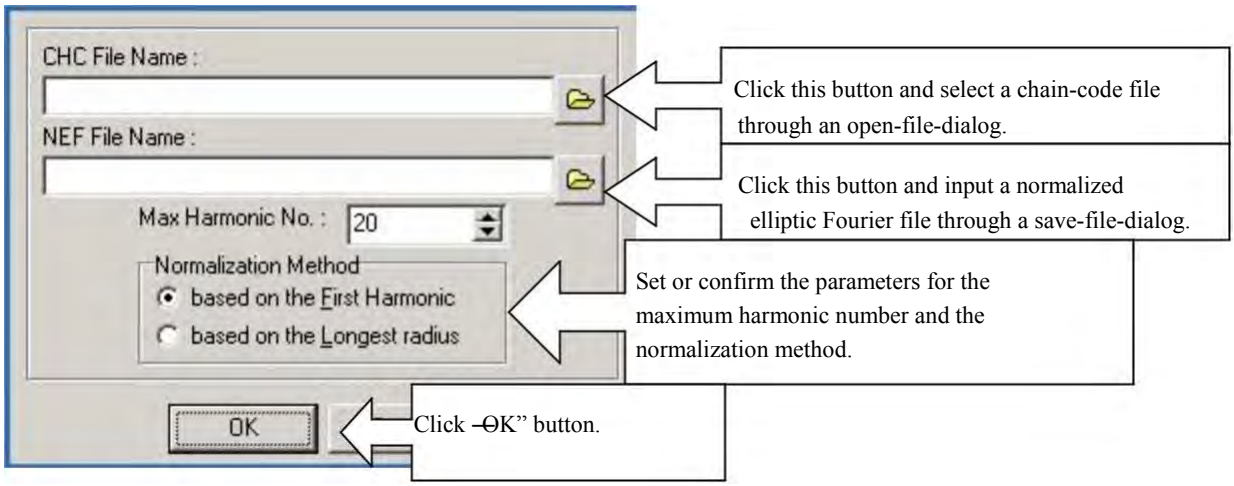


Fig. 19

16. Set analysis parameters as follows (A-D) (Fig. 19).

A) Click the button beside the –CHC File Name" box and select a chain-code file through an open file dialog. In this tutorial, please select —Sample_chc.chc" or —Test.chc" which will be obtained on the step 13 in this tutorial.

B) Click the button beside the —NEF File Name" box and input a normalized elliptic Fourier file through an save file dialog. If you select —Sample_chc.chc", the default name of the save file is set to —Sample_chc.nef".

C) Set —Max Harmonic No." as "20" and –Normalize Method" as —based on the First Harmonic". For detail information for these parameters, see the manual of SHAPE v.1.3.

D) Click –OK" button.

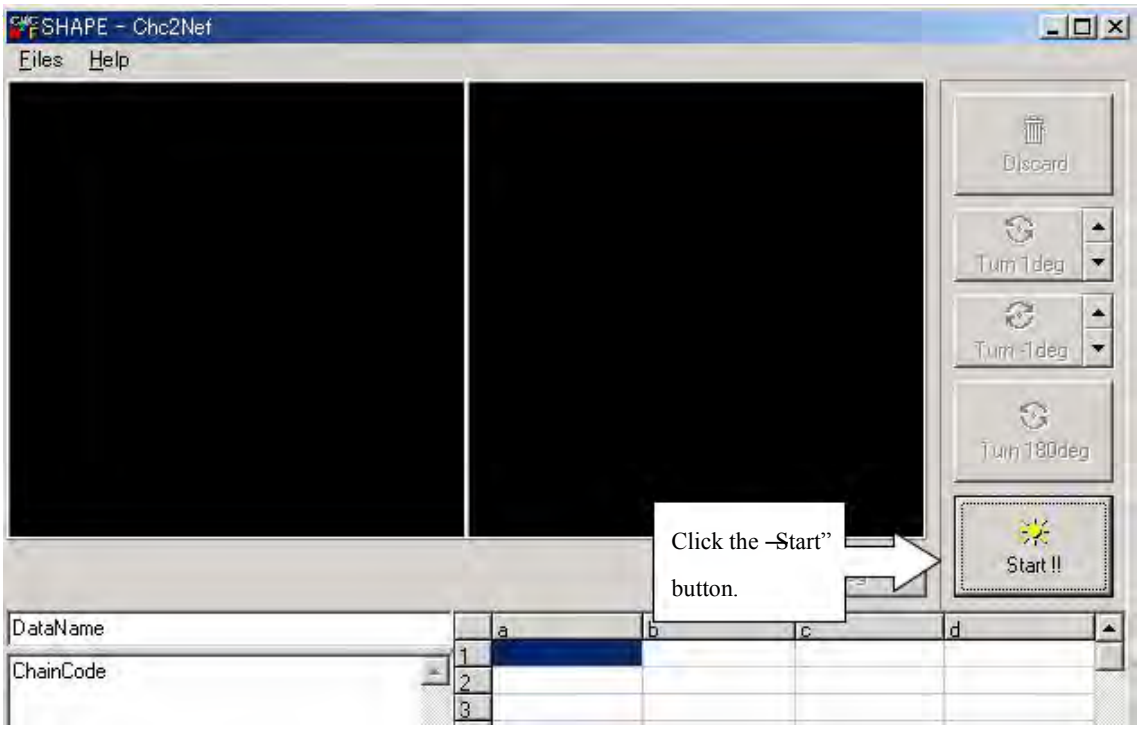


Fig. 20

17. Click the "Start" button (Fig. 20). The chain code of the first object is then converted to the normalized elliptic Fourier descriptors (NEFDs) and the NEFDs and the contour reconstructed by the NEFDs will be appeared in the window (Fig. 21)

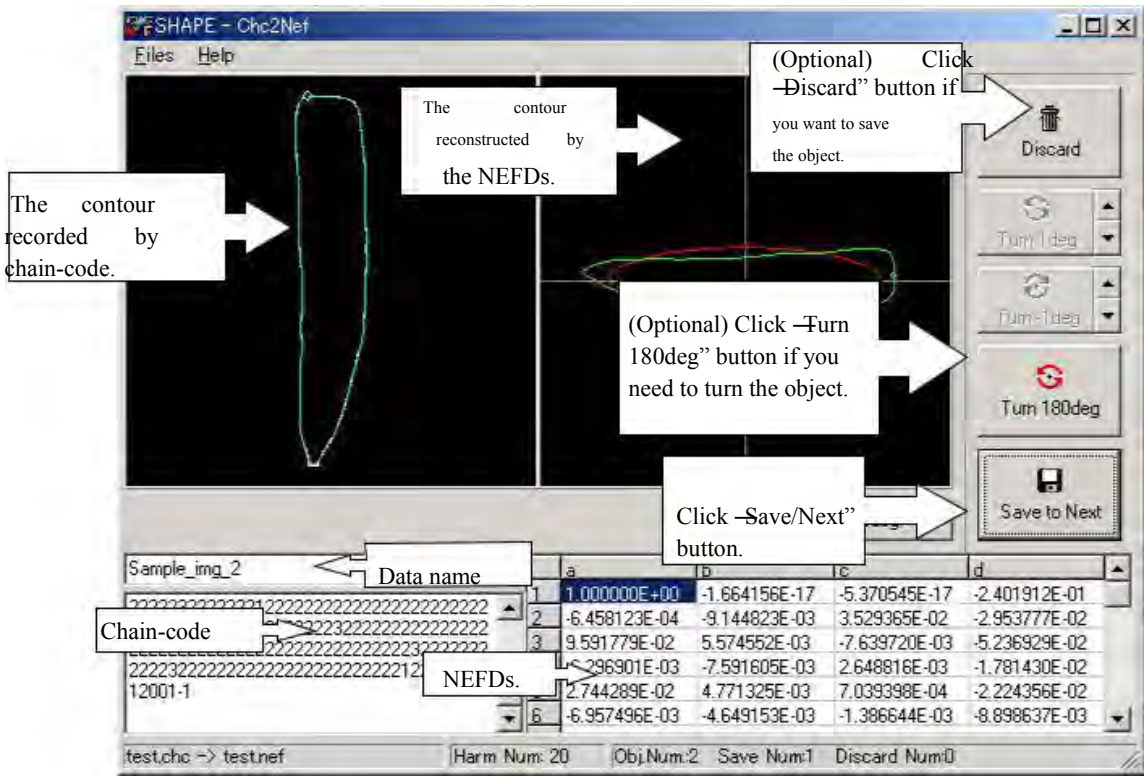


Fig. 21

18. (Optional) Click –Turn 180deg" button if you need to turn the object (Fig. 21).

19. (Optional) If you don't want to save the object, click the "Discard" button (Fig. 21).

20. Click —Save/Next" button. Then, the NEFDs obtained will be saved to the output file and the chain-code of the next object will be converted to normalized elliptic Fourier descriptors (Fig. 21). To convert the remaining objects, repeat the steps 18 and 19 until all the objects have been converted.

21. Close –Chc2Nef".

Final Step: Try out "PrinComp" and "PrinPrint"

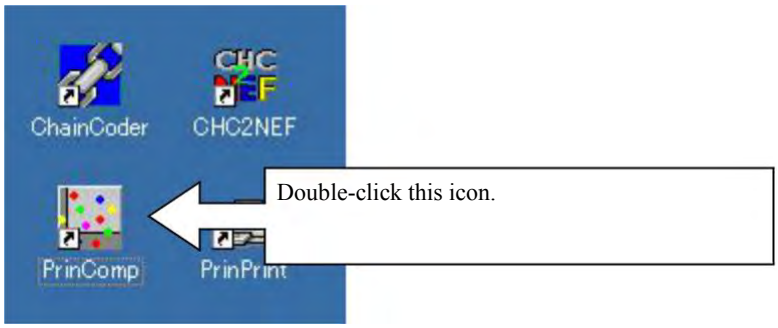


Fig. 22

22. Start –PrinComp" with double clicking the –PrinComp" icon (Fig. 22).

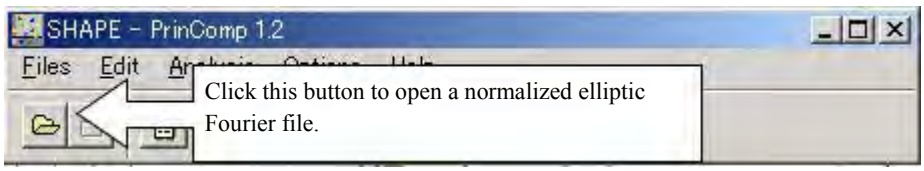


Fig. 23

23. Click —openFile" button (Fig. 23). An open-file-dialog will appear. Then select the sample file named —SampleNef.nef". (You can also select the normalized elliptic Fourier file obtained through this tutorial. In that case, the result of principal component analysis will, however, not be meaningful because the number of samples will be small for principal component analysis.)

24. After selecting the normalized elliptic Fourier (NEF) file, The "Nef File Information" window will appear. The "Number of Header Lines", "Number of Harmonics" and "Constant Coefficient" parameters are automatically set according to the information described in the header in the NEF file (Fig. 24).

Fig. 25

25. Click the analysis button to perform principal component analysis (Fig. 25) and –Principal Component Analysis Dialog" (Fig. 26) will be appeared.

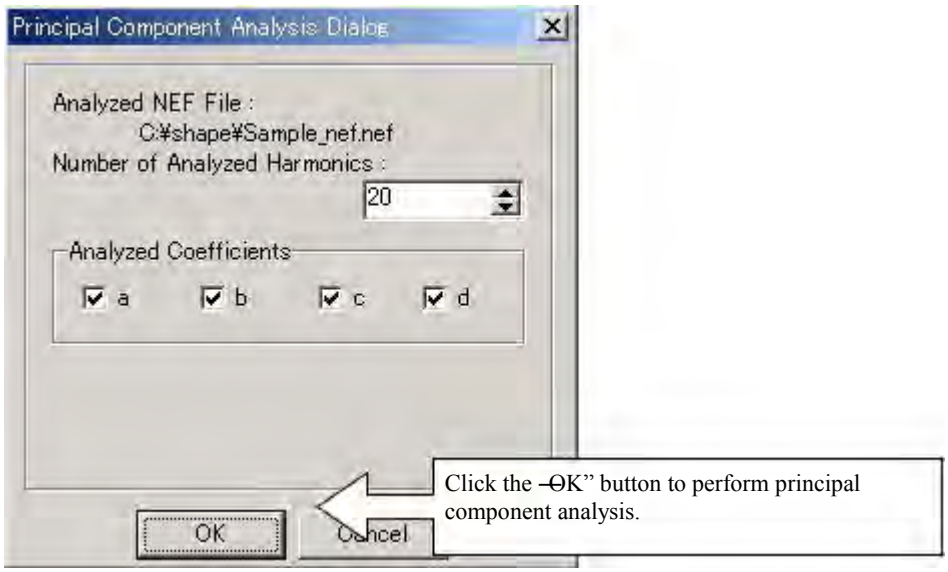


Fig. 26

26. Click –OK" button (Fig. 26), and the save file dialog will be appeared. After specifying the name of a saved file (*.pcr), principal component analysis will be performed and the information window which contains the result of principal component analysis will be appeared (Fig.27).

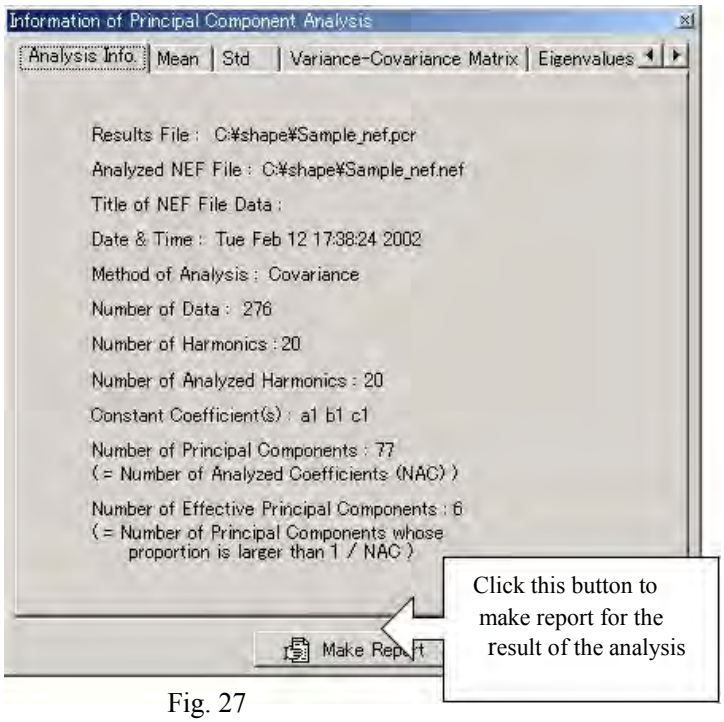


Fig. 27

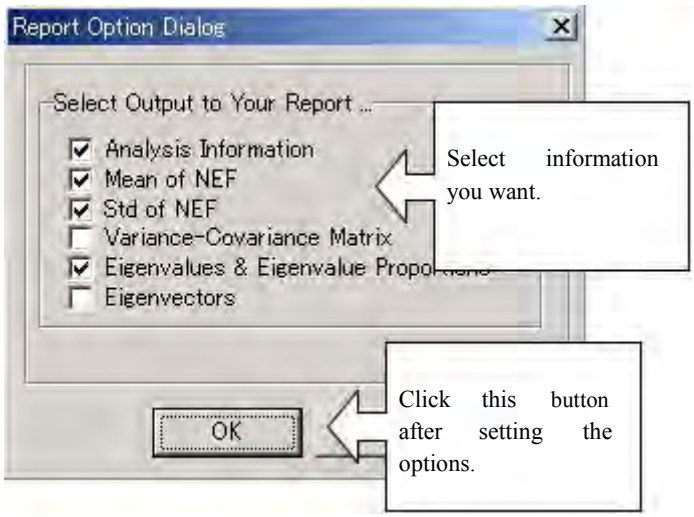


Fig. 28

27. Information contained in –Information of Principal Component Analysis" window can be output to a result report file in text format. Click —Make Report" button to make a result report (Fig. 27), and the —Report Option Dialog" will be appeared (Fig. 28). Set the options for the result report, and click the —OK" button. Then, the save dialog will be appeared. Input a file name to the dialog and click the "Save" button, a result will be saved in a report file

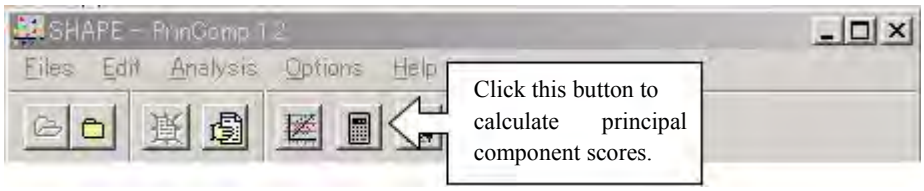


Fig. 29

28. To calculate the scores of principal components, select "Calculate Prin Score" button (Fig. 29). Then, the "Prin Score Dialog" will appear.

29.

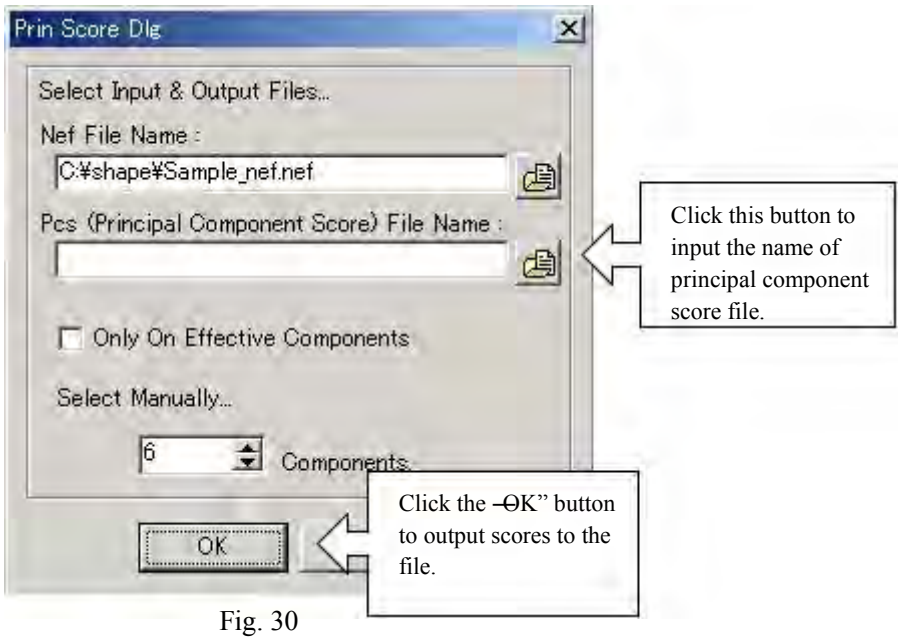


Fig. 30

29. Select the NEFDs file (which is automatically selected by the program) and input the principal component score file name (*.pcs) (this is done automatically by clicking the button beside the box) (Fig. 30). You can also change the number of the components for which the scores are calculated. Then, click the "OK" button. The file will then be saved in tabbed text format and can be opened as a Microsoft Excel worksheet for succeeding analysis from the appropriate (e.g. biological) perspective.

Fig. 31

30. To visualize the shape variation explained by each principal component, click the button with a line-drawing graphic (Fig. 31). After that, the "Reconstruct Contours Dialog" will be appeare.

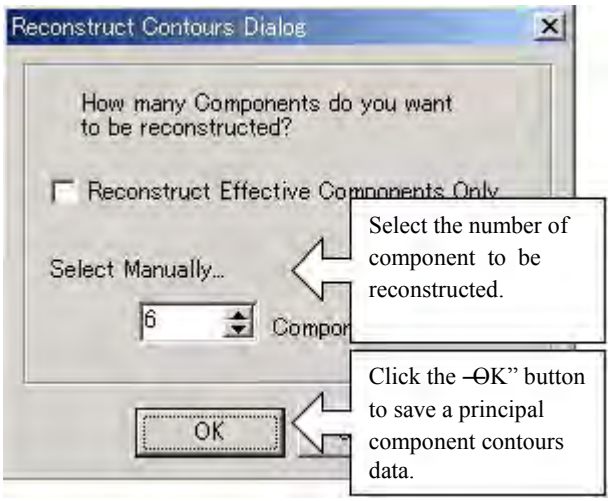


Fig. 32

31. Select the number of components to be reconstructed through the "Reconstruct Contours Dialog" (Fig. 32). Click the "OK" button, and a save dialog will appear. Input the name of a principal component contours file and click "Save". After that, —PrinPrint" will be automatically executed to display the result and the contours are displayed in a preview form for printing (Fig. 33). If —PrinPrint" cannot be automatically executed, start –PrinPrint" with double clicking the –PrinComp" icon and open the principal component contours file by selecting —Open" from –Files" menu.

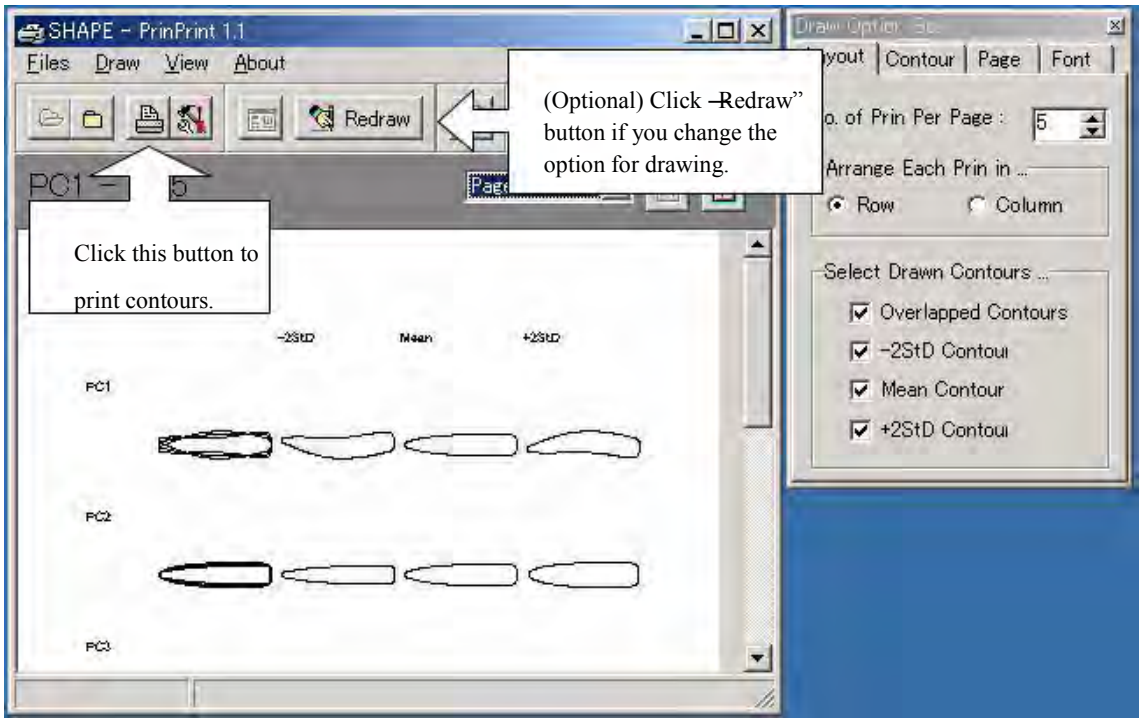


Fig 33

32. (Optional) You can change some options for drawing the contours, if desired. After setting the draw options, click the "Redraw" button (Fig. 33), the preview window will then be updated in accordance with the new settings.

33. Click the button with a printer icon (Fig. 33), and the print dialog will appear. After setting the printer properties, click the "OK" button. You can then print the contours.

34. Close "PrinComp" and "PrinPrint".

Optional Step: Try out "ChcViwer" and "NefViwer"

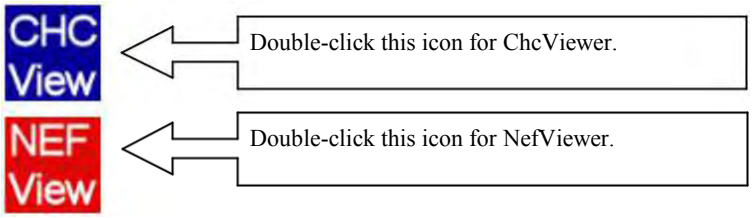


Fig. 34

35. Start "ChcViewer" (or "NefViewer") by double-clicking the "ChcViewer" (or "NefViewer") icon (Fig. 18).

A)

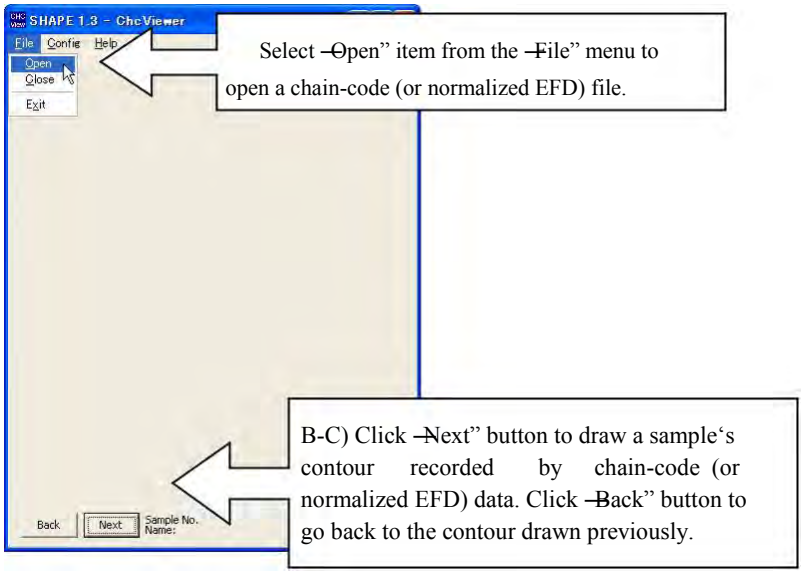


Fig. 35

36. View the sample's contour recorded by chain-code (or normalized EFD) data as follows (A-C) (Fig. 35).

A) Select "Open" from the "File" menu, and select a chain-code (or normalized EFD) file through an open file dialog. In this tutorial, please select "Sample_chc.chc" (or "Sample_nef.nef").

B) Click –Next" button. The contour of the first sample will appear in the window.

C) Click —Next" button to draw the contour of the next sample. Click "Back" button to go back to the previous sample.

The Museums and their importance

Vikas Pathak, B.B. Nayak, Layana P. and Shashi Bhushan
ICAR - Central Institute of Fisheries Education, off-Yari road, Versova, Mumbai

Introduction

–A museum is a non-profit, permanent institution in the service of society and its development, open to the public, which acquires, conserves researches, communicates and exhibit the tangible and intangible Heritage of Humanity and its environment for the purpose of Education, study and enjoyment" (International Council of museums, 2007). The non-profit institution or organization undertaking conservation research, education, training, documentation and other activities relating to museology are consider as museums.

Scope of museum

Scope of the museum is a stand-alone planning document of a museum that succinctly defines the purpose of its collection like why a museum need to collect something? Why museum need to preserve something? etc.. Scope of collection statement guides a museum in the acquisition and management, it is referenced in museums' resource planning, long range interpretive, planning documents that may affect the collection of museum object or their use, so these are the basics things that required to document in a proper way and this is considered as the scope of museum.

Basis of the determination of the scope of museum

The scope of a museum is depends upon the aims, mission, vision and objective of the museum. Aims of a museum based on that the museum personnel derive the scope of a museum, it also depends upon the nature of collection and related information such as research and documentation of object, designing education program, gallery and exhibition planning, periodic maintenance of object and developing museum publication all based on the all of this.

Necessity of the scope of museum document

It describe about the museum's collection section wise, helps to plan activities for the public, volunteers and donors management. It is the road map in evaluating new acquisition, budgeting, prioritizing resources, and overall planning and management of collection. Along with these things it helps museum to decide on selective acquisition for hands-on use and on deaccessioning, also helps in financial planning including manpower, cost curation, maintenance, storage, security, inventory control and energy cost.

What are Museums/Depository/Repositories?:

Museums: An institution that cares for (conserves) a collection of artifacts and other objects of artistic, cultural, historical, or scientific importance and makes them available for public viewing through exhibits that may be permanent or temporary.

Depository: Same meaning of repository (resting vs depositing). More used for financial deposits

Repository: A place where things are stored, usually for safe keeping or repositories are storage location of biological specimens in museums, herbaria, academic and research institutions, societies, etc. form critical infrastructure for research, education, regulation and legislation related to biodiversity. Provide the foundation for taxonomy and biodiversity science.

The depositions in the natural history museums and repositories reveal the great natural history and biodiversity of the nation and a source material for the taxonomists and biotechnologists to pursue their research

The function of museum

The prime function of a museum is to collect object which has historic scientific value and antique value so that that's why Museum is considered as the storehouse of object or the storehouse of wonder. it collects object through various modes like purchase, gift, loan, exchange, exploration, excavation, treasure, trove act, bequest etc., each museum authority adopts and publishes a written statement of its collection policy. Collection in museum depends upon the mission, vision and objectives of the museum and also on the needs of

research, education, conservation and preservation of evidences of natural and cultural heritage.

Next function is to preservation and conservation of this collected object, objects need regular care as every object is subject to deterioration due to spontaneous change in environment and subsequent physio-chemical factors. Regular care is required in both display and storage following the preventive conservation or curative conservation wherever needed, museum practices various preservation technique which are exclusively objects specific to protect them from attackes of organism like fungus. Insects, rodent etc and damage from humidity, temperature and light source and intensities, another important function of museum is to document the object which collected from various authentic sources. The research also act as important function because without research no museum can perform better for the public, better in display in preservation, storage and education so for everything means them need to research properly.

The exhibition is another essential function of museum because every museum exhibits object by forming permanent galleries or organising temporary.

The publication also act as important function of the museum, the Museum generally publishes two kind of materials one is popular publication and another one is scholarly. Publication of popular publication includes leaflet, folders, guide books etc., and in case of scholarly publication it published catalogues, journals, art albums, monographs etc.

Public services also a part of function of museum it has so many public services like it organizes various activities targeting specific group, science centers and museums run daily shows on the various areas of science and technologies in addition to the static exhibition, the service provides enjoyment entertainment and encourage.

Museums/repositories provide:

Overview of the rich biodiversity of the nature and acting as the reference guide for taxonomists**,** evidences for research**,** long term changes in ecosystems**,** ecological patterns, evolutionary and anthropogenic changes often go unseen and undocumented until samples from these collections materials are analysed and re-analysed using the latest technology

KEY MESSAGE: WITHOUT MUSEUMS/REPOSITORIES THERE CANNOT BE GROWTH OF TAXONOMY AND BIODIVERSITY SCIENCE.

Important scientific specimens are generally stored in collections where they serve as vouchers to document identification in published scientific research (applicable to research projects and surveys with public funding).

Who needs this information?

Parliamentarians, who need to ensure that laws will protect all biodiversity and that their legislation is directed at the top priorities; Field biologists, who need to identify the species with which they are working; Diplomats, who need to ensure that biodiversity-related conventions are meeting their conservation objectives; Agricultural scientists, who need to find species useful for integrated pest management (IPM), requiring a good understanding of species relationships; Customs and quarantine officials, who should be on the lookout for potentially invasive alien species; Eco-tourists, who want to identify the plants and animals they encounter in their travels; Planners, who need to carry out EIA for proposed projects that may affect biodiversity; Epidemiologists, who need to chart the distribution of diseases that may be transmitted between people and other animals.

Designated repositories of India

(Ministry of Environment and Forests, Government of India, order No. 26-15/2007-CSC dated 28.08.2008)

Sl. No.	Name of Institution	Category of biodiversity deposited	Type specimen
1.	Botanical Survey of India, Kolkata	Flora	Yes
2	National Bureau of Plant Genetic Resources, New Delhi	Plant genetic resources	
3	National Botanical Research Institute, Lucknow	Flora	Yes

4	Indian Council for Forestry Research and Education, Dehradun (Forest Research Institute, Dehradun; Institute of Forest Genetics and Tree Breeding, Coimbatore and Tropical Forest Research Institute, Jabalpur)	Flora and selected fauna such as termites, butterflies and moths)	Yes
5	Zoological Survey of India	Fauna	Yes
6	National Bureau of Animal Genetic Resources, Karnal, Haryana	Animal genetic resources	
7	National Bureau of Fish Genetic Resources, Lucknow	Fish	
8	National Institute of Oceanography, Goa	Marine flora and fauna	Type
9	Wildlife Institute of India, Dehradun	Faunal resources in protected areas	
10	National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan, UP	Microorganisms	Yes
11	Institute of Microbial Technology, Chandigarh	Microorganisms	Yes
12	National Institute of Virology, Pune	Viruses	Yes
13	Indian Agricultural Research Institute, New Delhi	Viruses/Fungi	Yes

Other Collections: Private institutions like Bombay Natural History Society and Universities with huge collections

Zoological Survey of India, Kolkata at glance

Faunal Holdings of ZSI

No	Centres	Types Specimens		Identified specimens	
		Vertebrates	Protists / Invertebrates	Vertebrates	Protists / Invertebrates
1	Andaman and Nicobar Regional Centre(ANRC), Port Blair	-	-	3807	18076
2	Arunachal Pradesh Regional Centre (APRC), Itanagar	-	-	1510	804
3	Central Zone Regional Centre (CZRC), Jabalpur	-	20	19085	41160
4	Estuarine Biology Regional Centre (EBRC), Gopalpur	-	02	11647	11149
5	North Eastern Regional Centre (NERC), Shillong	15	17	42824	44487
6	Freshwater Biology Regional Centre (FBRC), Hyderabad	-	-	1320	978
7	Gangetic Plains Regional Centre (GPRC), Patna	-	-	3389	3182
8	High Altitude Regional Centre (HARC), Solan	-	16	6939	16004
9	Marine Biology Regional Centre (MBRC), Chennai	-	-	27655	82280
10	Northern Regional Centre (NRC), Dehradun	10	49	47384	107841
11	Southern Regional	54	120	16043	102897
13	Western Regional Centre (WRC), Pune	1	1	14152	18340
14	Desert Regional Centre (DRC), Jodhpur	2	12	11218	
15	Zoological Survey of India Headquarters, Kolkata	17686		2.5 lakhs	
16	Marine Aquarium cum Regional Centre (MARC), Digha	Data not compiled			
17	Sunderban Regional Centre (SRC), Canning	Data not compiled			

Some Important museums of the world

A collection of bird specimens at the Museum of Comparative Zoology, Harvard University in Cambridge, Massachusetts

Collections of birds in the Zoological Museum in St Petersburg, Russia

The Oxford University Museum of Natural History

Vertebrate Zoology collections at the Smithsonian's National Museum of Natural History

St Petersburg : Zoological Museum

Museum fuer Naturkunde, Berlin: Wet collections

Kunming Natural History Museum, China

Western Australian Museum fish collection; 180,000 specimens of fishes from around 5000 species

Western Arctic Marine Fish Museum/Voucher Database / Arctic Marine Fish Distribution and Taxonomy

Megamouth on display at the Western Australian Museum - Maritime

67,000 specimens, the Grant Museum of Zoology, London

Indian Museum, Kolkata

Reference:

American Association of Museums. 2002. *Mastering Civic Engagement: A Challenge to Museums*. Washington, DC: American Association of Museums.

Bransford, J. D., A. L. Brown, and R. R. Cocking. 1999. *How People Learn.* Washington, DC: National Academy Press.

Dewey, J. 1916/1944. *Democracy and Education*. New York: McMillan.

Goode, G. B. 1888/1991. Museum history and museums of history. In *The Origins of Natural Science in America*, S. Kohlstedt, ed. Washington, DC: Smithsonian Press.

http://www.lesley.edu/faculty/ghein/papers_online/papers_online.html.

Hooper-Greenhill, E. 1999. *The Educational Role of the Museum, Second Edition*. London: Routledge.

Rennie, L. J. and D. J. Johnson. 2004. The nature of learning and its implications for research on learning from museums. *Science Education* 88: S4–S16.

Sullivan, L. E. and A. Edwards, eds. 2004. *Stewards of the Sacred.* Washington, DC: American Association of Museums.

Wetterlund, K. and S. Sayre. 2003. 2003 Art Museum Education Programs Survey, http://www.museum-ed.org/research/surveys/2019mused/index.shtml

Zeller, T. 1989. The historical and philosophical foundations of art museum education in America. In *Museum Education History, Theory, and Practice*, N. Berry and S. Mayer, eds. Reston, VA: The National Art Education Association.

		Morphometric and meristic measurements of fishes							
		Sample code							
		Location							
		Date							
		Barcode							
		Otoith /scale							
		Species							
Proportional measurements(%	1	Total length							
	2	Standard length							
	3	Head length							
	4	Pre-dorsal length							
	5	Pre-pelvic length							
	6	Pre-anal length							
	7	Pre-pectoral length							
	8	L. of 1st dorsal fin base							
	9	L. of 2nd dorsal fin base							
	10	Length of anal fin base							
Proportional measurement (% head length)	11	Diff. B/W upp. & low. Jaw							
	12	l. of preopercular spine u							
	13	L							
	14	Pre-orbital length							
	15	Post- orbital length							
	16	Snout length							
	17	Maximum eye dia.							
	18	Inter orbital width							
	19	Maxi. L. of 1st dorsal spine							
	20	Maxi. L. of 2st dorsal spine							
	21	Maxi. L. of 2nd dorsal ray							
Counts	22	No. of scale above the L. L							
	23	No. of 1st dorsal fin spine							
	24	No. of 2nd dorsal fin ray							
	25	No. of Anal fin ray							
	26	No. of pectoral fin ray							
	27	No. of pelvic fin ray							
	28	Gill rakers Up.							
	29	lower							
	30	No. l. line scale							
	31	Pored lateral line scale							

Notes :

ICAR-CIFE HQ & Centres

Kolkata Centre

32, G. N. Block, Sector-V,
Salt Lake City, Kolkata,
West Bengal – 700 091
kolkata@cife.edu.in
Ph.: 033 - 2357 3893
Fax: +91 033-2357 5269

Kakinada Centre

Near Old Burma Shell,
Beach Road,
Kakinada, Andhra Pradesh
Pin 533 007
kakinada@cife.edu.in
Ph.: 0884-2376746
Fax: +91 0884-2373602

Rohtak Centre

Lahli, Via Anawal,
Rohtak – 124 411, Haryana
rohtak@cife.edu.in
Ph.: 01258 – 253595 / 253506
Fax: +91 01258-253506

Powarkheda Centre

Powarkheda, Dist. Hoshangabad,
Madhya Pradesh- 461 110
powarkheda@cife.edu.in
Ph.: 07574-227280
Fax: +917574 227208

Motipur Centre

C/o Regional Centre of Indian Institute
of Sugarcane Research
Motipur, Distt. Muzaffarapur, Bihar
Pin 843 111,
motipur@cife.edu.edu.in
Ph.: 9004864708

ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(University under Sec.3 UGC Act, 1956)
Indian Council of Agricultural Research
Panch Marg, Off Yari Road,
Mumbai - 400 061
+91 22 263636404 / 26374306
www.cife.edu.in

CIFE's Research Vessel MFV Saraswati