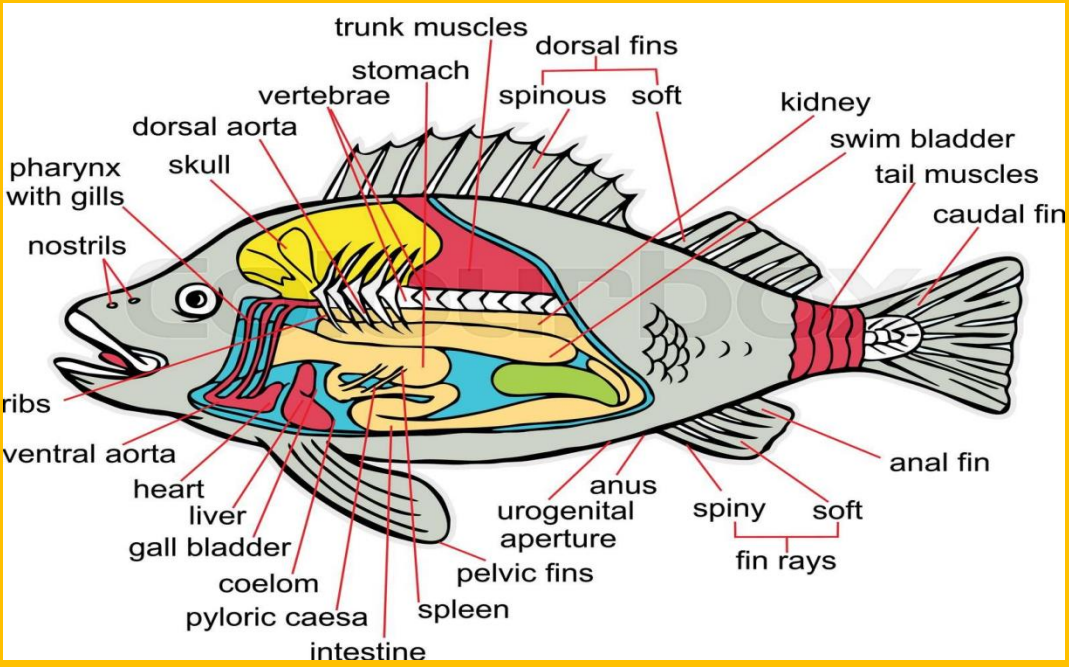
MSCZO-608L

M.Sc. IV Semester

LABORATORY EXERCISE



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LABORATORY EXERCISE

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Course: Laboratory Exercise
Course code: MSCZO608L **Credit: 3**

UNIT 01: FISH ECO-BIOLOGY

CONTENTS

1.1OBJECTIVES

We will understand in this topic about the Skeletons of Cyprinoid and Siluroid fish, Taxonomic studies of fresh water fishes, Observation of length, weight and Length-weight relationship. Determination of age & growth. Permanent preparation of scales, sensory, ampullae etc. Morphometry & Histology of fish body and organs. Microscopic study of fish parasite, pathogens, Fungi and pathogenic bacteria. Study of pituitary gland & preparation of PGE.Study of respiratory organs of fish. Study of reproductive organs of fish. Study of nervous & sensory organs of fish.

1.2 INTRODUCTION

The ecology of fishes is extremely varied due to the great diversity of species within the five fish classes. Habitats range from regions deep within the sea to lakes and streams high on mountaintops. Most fish are predators, but the nature of their prey and how they consume that prey varies. In this section we will consider general aspects of the ecology of each class of fish. Hagfish are deep-sea bottom dwellers. These fish are predators that feed on other fish by entering their bodies through an orifice, such as the mouth or anus, and consuming them from the inside. Small invertebrates also make up a large part of their diets. Because of their remote habitats, there is not a lot known about the ecology of hagfish. It is likely that their predatory role as well as their position as prey for other predatory fishes in the food chain is important in maintaining ecological balance.

1.3SKELETONS OF CYPRINOID AND SILUROID FISH

The osteology (study of bones) of fishes is more com plicate than in other vertebrates because fish skeletons are made up of many more bones. For example, humans (sarcopterygian) have 28 skull bones, a primitive reptile (sarcopterygian) has 72, and a fossil chondrostean (actinopterygian) fish more than 150 skull bones (Harder 1975). The general evolutionary trend from primitive actinopterygians to more advanced teleost's and from aquatic sarcopterygians totetrapods has been toward fusion and reduction in number of bony elements (Trends during teleosteanphylogeny).

Why do we need to know about the osteology of fishes? First of all, we cannot really understand such processes as feeding, respiration, and swimming without knowing which jaw bones, branchial bones, and fin supports are involved. Knowledge of the skeleton is necessary to understand the relationships of fishes and much of classification is based on osteology. Identification of bones is also important in paleontology, in identifying food of predatory fishes, and in zoo archeology for learning about human food habits from kitchen midden material

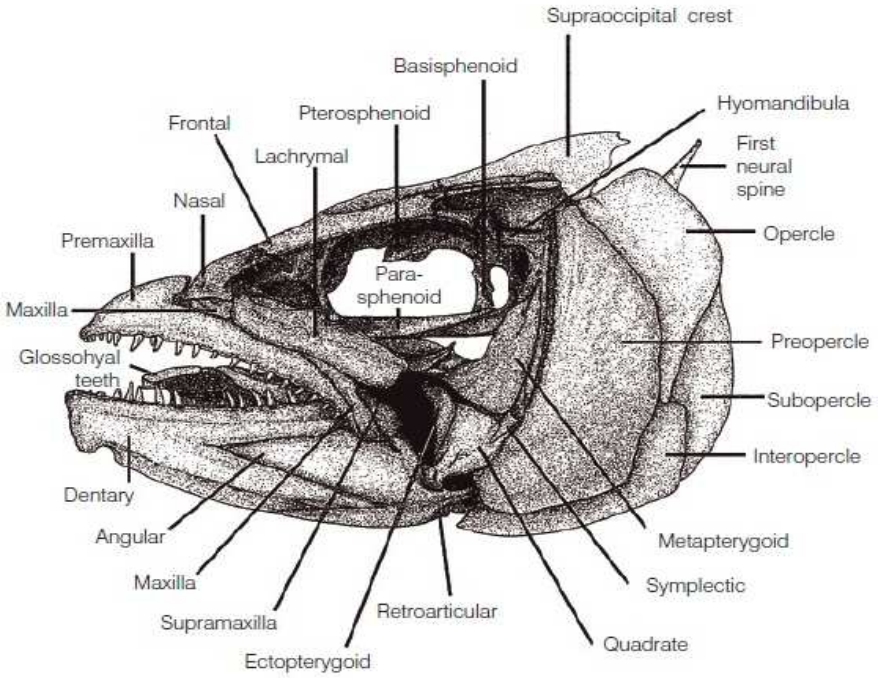

Fig.1.1Skull of Doftooth Tuna (Gymnosarda unicolor)

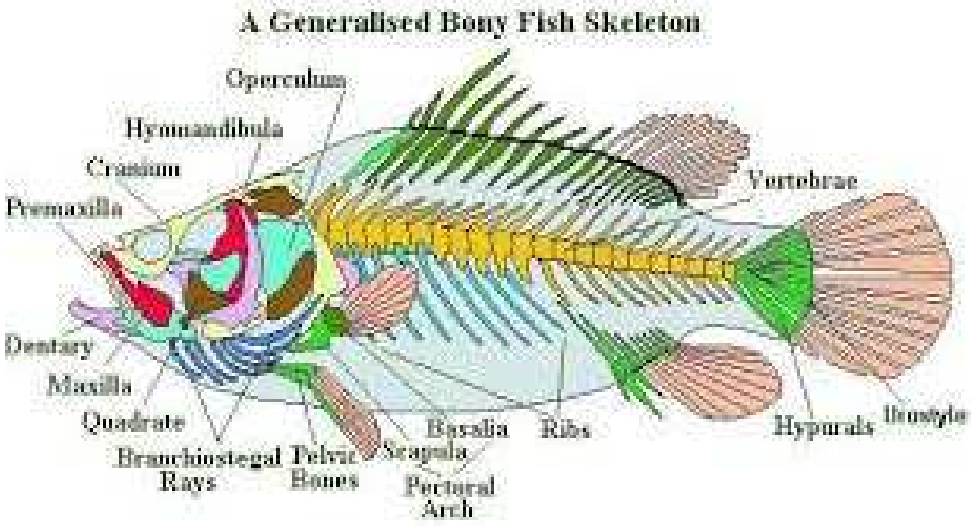

Fig.1.2

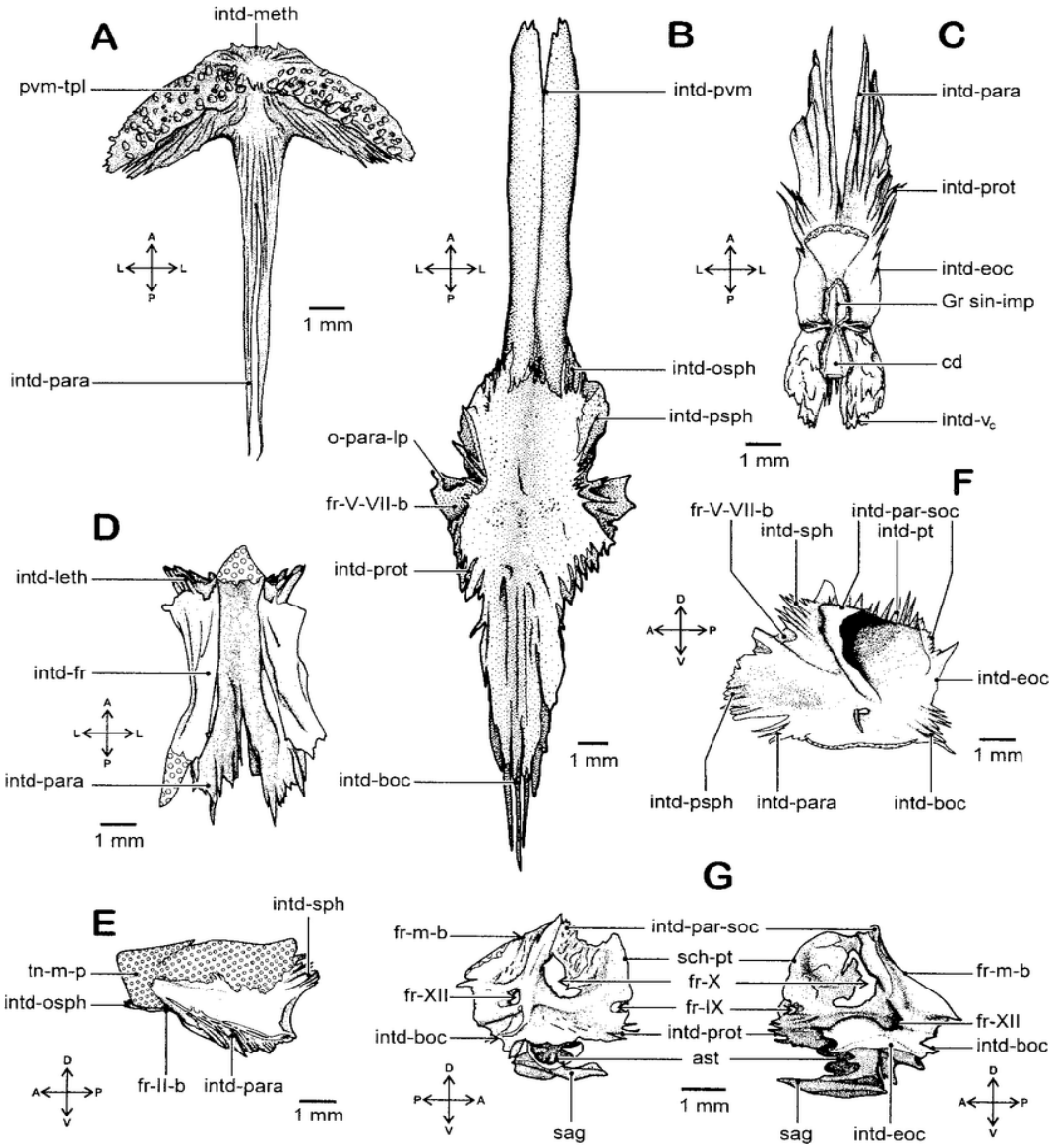

Fig.1.3Skull floor bones of juvenile Clarias gariepinus

1.4TAXONOMIC STUDIES OF FRESH WATER FISHES

Freshwater fish are those that spend some or all of their lives in fresh water, such as rivers and lakes, with a salinity of less than 1.05%. These environments differ from marine conditions in many ways, especially the difference in levels of salinity. To survive fresh water, the fish need a range of physiological adaptations.

41.24% of all known species of fish are found in fresh water. This is primarily due to the rapid speciation that the scattered habitats make possible. When dealing with ponds and lakes, one might use the same basic models of speciation as when studying island biogeography.

Freshwater fish differ physiologically from saltwater fish in several respects. Their gills must be able to diffuse dissolved gases while keeping the salts in the body fluids inside. Their scales reduce water diffusion through the skin: freshwater fish that have lost too many scales will die. They also have well developed kidneys to reclaim salts from body fluids before excretion.

Many species of fish do reproduce in freshwater, but spend most of their adult lives in the sea. These are known as anadromous fish, and include, for instance, salmon, trout, sea lamprey and three-spined stickleback. Some other kinds of fish are, on the contrary, born in salt water, but live most of or parts of their adult lives in fresh water; for instance the eels. These are known as catadromous fish.

Species migrating between marine and fresh waters need adaptations for both environments; when in salt water they need to keep the bodily salt concentration on a level lower than the surroundings, and vice versa. Many species solve this problem by associating different habitats with different stages of life. Eels, anadromous salmoniform fish and the sea lamprey have different tolerances in salinity in different stages of their lives.

Among fishers in the United States, freshwater fish species are usually classified by the water temperature in which they survive. The water temperature affects the amount of oxygen available as cold water contains more oxygen than warm water.

Coldwater

Coldwater fish species survive in the coldest temperatures, preferring a water temperature of 50 to 60 °F (10–16 °C). In North America, air temperatures that result in sufficiently cold water temperatures are found in the northern United States, Canada, and in the southern United States at high elevation. Common coldwater fish include brook trout, rainbow trout, and brown trout. Coolwater fish species prefer water temperature between the coldwater and the long warm water species, around 60 to 80 °F (16–27 °C). They are found throughout North America except for the southern portions of the United States. Common cool water species include muskellunge, northern pike, walleye, and yellow perch.

Warm water

Warm water fish species can survive in a wide range of conditions, preferring a water temperature around 80 °F (27 °C). Warm water fish can survive cold winter temperatures in northern climates, but thrive in warmer water. Common warm water fish include catfish, largemouth bass, bluegill, crappies, and many other species from the family Centrarchidae.

In 2021, a group of conservation organizations estimated that one-third of the world's freshwater fish species were at risk of extinction. A global assessment of freshwater fishes estimates an average decline of 83% in populations between 1970 and 2014. The protection of 30% of Earth's surfaces by 2030 may encompass freshwater habitat and help protect these threatened species.

There is an increasing trend in freshwater fish for local taxonomic, functional, and phylogenetic richness in more than half of the world's rivers. This increase in local diversity is primarily explained by anthropogenic species introductions that compensate for or even exceed extinctions in most rivers.

HABITAT DESTRUCTION

Intentional anthropogenic reconstruction and rerouting of waterways impacts stream flow, water temperature, and more, impacting normal habitat functionality. Dams not only interrupt linear

water flow and cause major geological channel shifts, but also limit the amount of water available to fishes in lakes, streams and rivers and have the potential to change the trophic structure because of these alterations of the habitat and the limitations to movement and connectivity.

Unnatural water flow below dams causes immense habitat degradation, reducing viable options for aquatic organisms. Upstream migration is hindered by the dam structure and can cause population declines as fishes don't have access to normal feeding and/or spawning grounds. Dams tend to affect upstream species richness, that is, the number of fish species in the ecological community. Additionally, dams can cause the isolation of fish populations, and the lack of connectivity creates possible problems for inbreeding and low genetic diversity. The loss of connectivity impacts the structure of community assemblies and increases the fragmentation of habitats, which can compound existing problems for vulnerable species.

Temperature alterations are another unintended consequence of dam and land use projects. Temperature is a very important part of aquatic ecosystem stability, and thus changes to stream and river water temperature can have large impacts on biotic communities. Many aquatic larvae use thermal cues to regulate their life cycles, mostly notably here, insects. Insects are a large part of most fish diets, so this can pose a great dietary problem. Temperature can cause changes in fish behavior and distribution habits as well by increasing their metabolic rates and thus their drive to spawn and feed.

Linear systems are more easily fragmented and connectivity in aquatic ecosystems is vital. Freshwater fishes are particularly vulnerable to habitat destruction because they reside in small bodies of water which are often very close to human activity and thus easily polluted by trash, chemicals, waste, and other agents which are harmful to freshwater habitats.

Land use changes because major shifts in aquatic ecosystems. Deforestation can change the structure and sedimentary composition of streams, which changes the functionality of the habitat for many fish species and can reduce species richness, evenness, and diversity. Agriculture, mining, and basic infrastructural building can degrade freshwater habitats. Fertilizer runoffs can create excess nitrogen and phosphorus which feed massive algae blooms that block sunlight, limit water oxygenation, and make the habitat functionally unsustainable for aquatic species. Chemicals from mining and factories make their way into the soil and go into streams via runoff.

More runoff makes its way into streams since paved roads, cement, and other basic infrastructure do not absorb materials, and all the harmful pollutants go directly into rivers and streams. Fish are very sensitive to changes in water pH, salinity, hardness, and temperature which can all be affected by runoff pollutants and indirect changes from land use.

EXOTIC SPECIES

An exotic (or non-native) species is defined as a species that does not naturally occur in a certain area or ecosystem. This includes eggs and other biological material associated with the species. Non-native species are considered invasive if they cause ecological or economic injury.

The introduction of exotic fish species into ecosystems is a threat to many endemic populations. The native species struggle to survive alongside exotic species which decimate prey populations or outcompete indigenous fishes. High densities of exotic fish are negatively correlated with native species richness. Because the exotic species was suddenly thrown into a community instead of evolving alongside the other organisms, it doesn't have established predators, prey, parasites, etc. which other species do, and the exotic species thus has a fitness advantage over endemic organisms.

One such example is the destruction of the endemic cichlid population in Lake Victoria via the introduction of the predatory Nile perch (*Lates niloticus*). Although the exact time is unknown, in the 1950s the Ugandan Game and Fisheries Department covertly introduced the Nile perch into Lake Victoria, possibly to improve sport fishing and boost the fishery. In the 1980s, the Nile perch population saw a large increase which coincided with a great increase in the value of the fishery. This surge in Nile perch numbers restructured the lake's ecology. The endemic cichlid population, known to have around 500 species, was cut almost in half. By the 1990s, only three species of sport fish were left to support the once multispecies fishery, two of which were invasive. More recent research has suggested that remaining cichlids are recovering due to the recent surge in Nile perch commercial fishing, and the cichlids that are left have the greatest phenotypic plasticity and are able to react to environmental changes quickly.

The introduction of the rainbow trout (*Oncorhynchus mykiss*) in the late 19th century resulted in the extinction of the yellow fin cutthroat trout (*Oncorhynchus clarkii macdonaldi*) found only in the Twin Lakes of Colorado, USA. The yellow fin cutthroat trout was discovered in 1889 and was recognized as a subspecies of the cutthroat trout (*Oncorhynchus clarkii*). The rainbow trout

was introduced to Colorado in the 1880s. By 1903, the yellow fin cutthroat trout stopped being reported. It is now presumed extinct. The rainbow trout is invasive worldwide, and there are multiple efforts to remove them from their non-native ecosystems.

Both species are among the "100 of the World's Worst Invasive Alien Species," as determined by the IUCN Invasive Species Specialist Group based on their effect on anthropogenic activities, environmental biodiversity and their ability to act as a case study for important ecological issues.

1.5OBSERVATION OF LENGTH, WEIGHT AND LENGTH-WEIGHT RELATIONSHIP

Standard weight in fish is the typical or expected weight at a given total length for a specific species of fish. Most standard weight equations are for freshwater fish species.

Weight-length curves are developed by weighing and measuring samples of fish from the population. Methods of obtaining such samples include creel surveys, or measurements of fish caught by commercial fishermen, recreational fishermen and/or by the researchers themselves. Some scientists use cast nets, trotlines, or other means to catch many individual fish at once for measurement. To determine a standard weight equation, several data sets or weight-length relationships representing a species across its range are used.

As fish grow in length, they increase in weight. The relationship between weight and length is not linear. The relationship between length (L) and weight (W) can be expressed as:

$$W = a\,L^{b}$$

When the equation is for standard weight, the standard weight for a given length is written as W_s. The exponent b is close to 3.0 for most species. The coefficient a varies between species. If the exponent b is greater than three for a certain fish species, that species tends to become relatively fatter or have more girth as it grows longer. For largemouth bass, the value of b is 3.273. If the exponent b is less than three for a certain fish species, that species tends to be more streamlined. For burbot, the value of b is 2.898. While the standard weight for a largemouth bass that is 500 mm long is about 2 kg, the standard weight for a burbot that is 500 mm long is only about 0.9 kg.

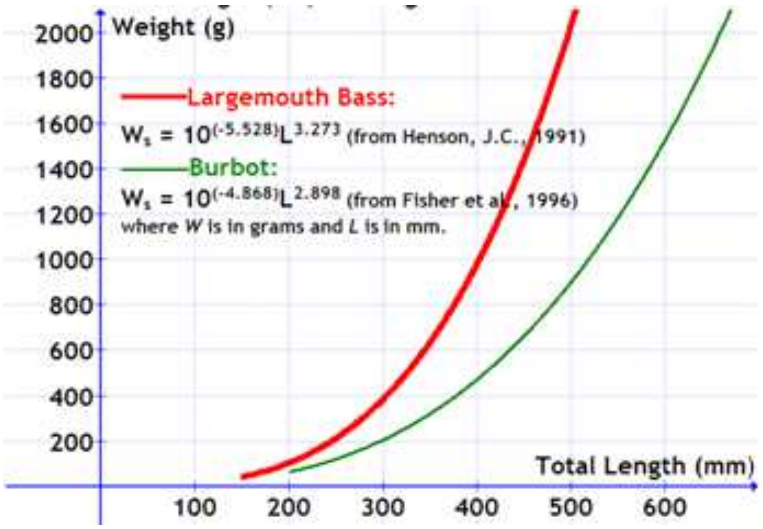

Fig.1.4 Standard weight (WS) for largemouth Bass and Burbot

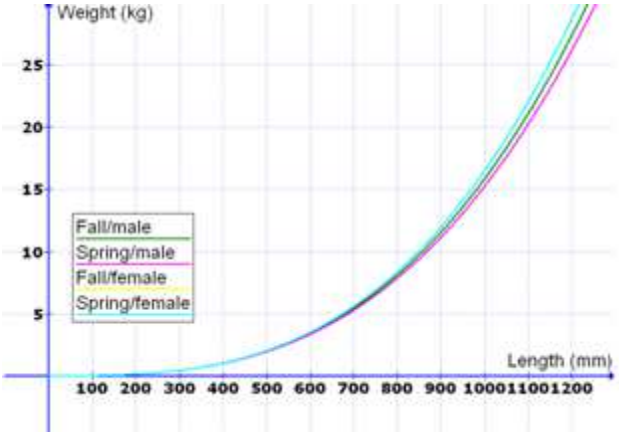

Fig.1.5 Weight V/s Length

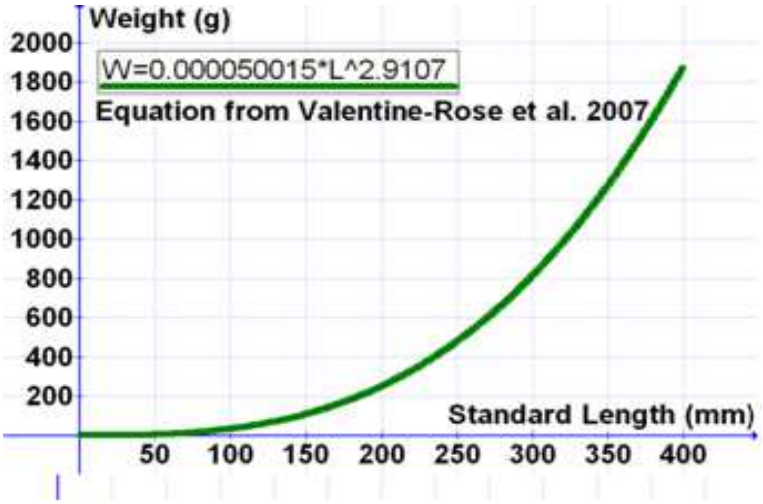

Fig.1.5 Weight V/s Standard Length

Standard weight curves are often based on the 75th percentile weight data rather than the average of all the data available. Murphy et al. (1991) suggested that it is preferable that standard weight equation represents the entire geographical range of a species, and that they be used for comparison purposes rather than management targets. Practically, weight-length equations are often developed for sub-populations from specific geographic areas, but these are different from standard weight relationships.

FACTORS AFFECTING THE STANDARD WEIGHT

Length measurements reported for fish may be of the fish's total length, fork length, or maximum standard length. For standard weight equations, the total length is used.

In some species, male and female fish have different standard weight curves. For example, Anderson and Neumann report different standard weight equations for male and female paddlefish. Some researchers have also reported separate standard weight equations when a species has lentic (living in still water) and lotic (living in flowing water) populations. For example, separate standard weight equations have been published for lentic and lotic rainbow trout.

APPLICATION

Standard weight is used as a basis for comparison to assess the health of an individual or group of fish. Generally, fish that are heavier than the standard weight for their length are considered healthier, having more energy reserves for normal activities, growth and reproduction.

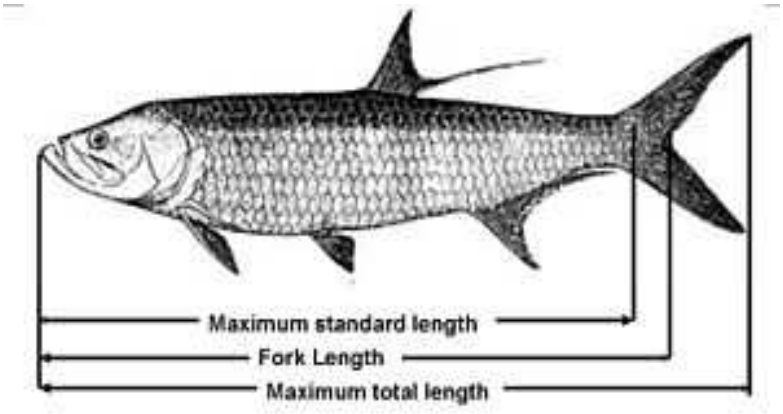

Fig.1.6

Fish may weigh less than expected for their length for many reasons, and a scientist must consider more information before assigning a cause. One of the simplest reasons is lack of food/prey. Lack of prey in turn could be the result of overpopulation of the predator, for example, competition from another predator species, unsuitability of the environment for reproduction of the prey, or dying of the prey for some reason. A fish may also weigh less than expected due to a change in activity level or metabolism due to some environmental factor.

Standard weight equations, together with some measure of a fish's condition, can be used in aquaculture to measure the effectiveness of various feeding, temperature control, containment or other practices. The actual measure of a fish's condition using standard weight is done different ways.

The relative weight (W_r) of an individual fish is its actual weight divided by its standard weight, times 100%. A fish of "normal" weight has a relative weight of 100 percent. The relative weight of a fish does not indicate its health on a continuous scale from 0 -100%, however. For example, Simpkins et al. found that juvenile rainbow trout with a condition index of less than 80% were at a high risk of dying. Relative weight is one of several common measures of condition used in fisheries assessment and management.

Fulton's condition factor, K, is another measure of an individual fish's health that uses standard weight. Proposed by Fulton in 1904, it assumes that the standard weight of a fish is proportional to the cube of its length:

$$\mathbf{K = 100(W/L^3)}$$

Where *W* is the whole body wet weight in grams and *L* is the length in centimeters; the factor 100 is used to bring K close to a value of one.

1.6 DETERMINATION OF AGE & GROWTH

Knowledge of fish age characteristics is necessary for stock assessments, and to develop management or conservation plans. Size is generally associated with age; however, there are variations in size at any particular age for most fish species making it difficult to estimate one

from the other with precision. Therefore, researchers interested in determining a fish age look for structures which increase incrementally with age. The most commonly used techniques involve counting natural growth rings on the scales, otoliths, vertebrae, fin spines, eye lenses, teeth, or bones of the jaw, pectoral girdle, and opercular series. Even reliable aging techniques may vary among species; often, several different bony structures are compared among a population in order to determine the most accurate method.

Fig.1.7 Removing Otolith to determine fish age

ANALYSIS OF AGES

Not long after Hoffbauer's and Reibisch's findings were published, aging was used in fishery assessments of the early 1900s. One of the first to focus on the applications of fish aging was the Norwegian fisheries scientist Johan Hjort. Focusing on fish scales, Hjort developed an extensive aging program collecting statistics on birth rate, age-distribution and migration. Hjort's research elicited debate from the biomathematician D'Arcy Wentworth Thompson, who later rescinded his criticisms. His research otherwise received glowing praise and would lead to fundamental changes in the way fish populations were studied and managed.

AGING STRUCTURE & TECHNIQUES

SCALES

Scales are the most widely used aging structure in North America because of their non-lethal ease of collection. Counting the number of annuli (rings) on a scale provides the fish age and the spacing between rings is proportional to the growth of the fish. The ease of collection of this aging structure is not without its tradeoffs, as the major bias of scales used as an age estimation structure is their tendency to underestimate the age of older fish.

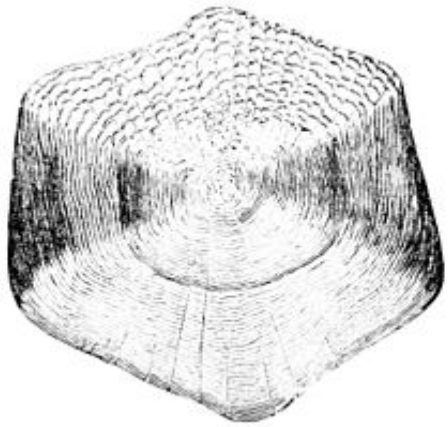

Fig.1.8

OTOLITHS

Fish otoliths are the earbones of a teleost (bony) fish and are present in pairs; fish have three pairs, the lapilli, the sagittae, astersci. These three pairs of otoliths in teleost fishes differ in form, function, size, shape, and ultra structure. Otoliths function in fishes' hearing, equilibrium, and acceleration. Otolith micro structural studies exist for 50 families and 135 species of fish and squid.The size and shape of otoliths vary widely depending on the species. Without prior experience it is difficult to predict the exact size, shape, and position of a given species. There is also interspecies variation, especially ontogenetic changes as a fish experiences growth. Otoliths are generally easier to read than scales and are more accurate, being internal and never reabsorbing like scales. Often the sagittae are analyzed for growth as they are the largest of the three otoliths and therefore easiest to remove. When preparing to analyze otoliths, generally if

the otolith is <300 mm then it can be analyzed intact, when >300 mm otoliths contain too much three-dimensional material and must be sectioned to analyze it more clearly. The steps to preparing otoliths are to 1. Embed or mount the otolith 2. Section and polish 3. Store the otolith section safely.

CALCIFIED OR BONY STRUCTURES

The choice of calcified or bony structures for aging varies among species, a structure used in one species may not be the same structure used in another. Not all bony structures lay down growth rings equally. Such bony structures used for age estimation are vertebrae, opercula, fin rays, pectoral spines, among others. Bony structures are often compared to otoliths as far as accuracy. Some bony structures such as fin rays and pectoral spines may be harvested without sacrificing the specimen, unlike otoliths. Preparation for bony parts involves first cleaning by soaking the structure in bleach or boiling to remove soft tissues. Depending on the size, shape, and structure of the calcified aging part it may be examined whole or more likely, sectioned. Estimation of annuli is similar to that of otoliths.

ANALYZING AGES CLASS STRUCTURE

Fish ages are often examined along with measurements of length and weight which combined can provide information on stock composition, age at maturity, life span, mortality, and production. Other purposes of performing age structure analysis are growth analysis, population dynamics estimates and resource management. Data from a particular study can delineate individuals into specific age classes. Exploited species often have the older, larger individuals removed from the population because they are the first removed by fishers leaving the younger smaller individuals. This effect may have serious consequences for that population. By performing age analysis studies we can identify these types of effects as well their implications to the status of the population.

Age structure analysis can be performed by the above methods which are the most direct, through estimates of length and weight, or a combination of both. Once the data is acquired and individuals are arranged in their respective age classes, one can attempt to attribute trends to the age distribution. For example, in Jaurequizar and Guerrero (2009), the researchers were examining the age structure of a population as a function of a period of four years which

experienced varying environmental conditions (two average years to El Niño and La Niña years). The dominant age classes were affected by the environmental conditions.

While the age analysis has been around in some form for over 250 years, there has only more recently been a rapid advance in techniques and uses of this information. There are still efforts required to further validate these aging methods and to determine new techniques. As the population of the world's fish continues to decline due to exploitation age structure analysis data will only become more important as we to try understand multiple effects on population dynamics.

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UNIT 2 PERMANENT PREPARATION

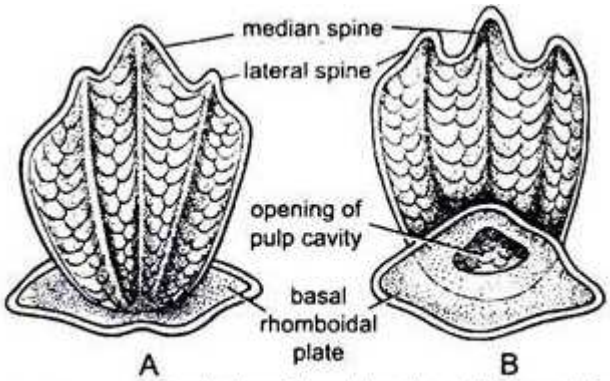
2.1 PERMANENT PREPARATION OF SCALES, SENSORY, AMPULLAE ETC

Study of placoid, cycloid and ctenoid scales through permanent slides

1. Placoid Scale of Shark: Placoid scales are found in elasmobranchs.

PREPARATION: Cut a small piece of skin from the dorsal surface of a shark (Scoliodon sp.) Put it in a hard glass test tube containing 5 to 10% KOH solution. Boil with constant stirring till the skin dissolves. Pour the contents of the test tube in a large watch glass. Allow to cool. The scales settle at the bottom. Decant the fluid. Repeat decantation with water till the last trace of KOH is removed. Pipette a drop of water with the scales and put it on a slide. Remove the water with a piece of blotting paper and mount in glycerine. Staining, if required should be done in a small watch glass. Mount following routine procedure.

STRUCTURE:



Scoliodon. Placoid scales. A–Dorsal view; B–Ventral view.

Fig.2.1

i. The scale has a base and a body. ii. The basal plate is somewhat diamond shaped with a pulp cavity on the ventral surface, at the centre.

iii. The proximal end of the body attached to the basal plate is narrow. It widens distally. iv. A few spines are present in the body which project a little beyond the distal margin.

For Scoliodon fish slide (**Placoid Scales**) Take a few small pieces of the skin of Scoliodon and boil in 5 to 10% KOH solution in test tube till skin dissolves. Cool and allow the scales to settle at bottom. Decant KOH and wash the material in water several times to remove KOH. Stain in borax carmine or picro-indigo carmine, dehydrate, clear and mount. Placoid scales do not take stains properly. These scales can be mounted without Staining.

Comments:

(1) Placoid scales or odontoids are minute dermal denticles, closely arranged in regular oblique rows.

(2) They form entire exoskeleton of the shark and give a rough appearance to the skin. (3) Each

placoid scale comprises of a diamond-shaped, basal plate embedded in the skin and is derived from dennis.

(4) Anteriorly the scale has a flat trident spine projecting out of the skin.

(5) With very few exceptions, placoid scales are abundantly found in dermis of elasmobranch fishes.

(6) Placoid scales are arranged in regular oblique rows. They are dermal in origin and cover entire surface of the body, forming dermal exoskeleton of the sharks.

(7) Each scale is composed of a basal bony plate embedded in the dermis, from which projects trident spines.

(8) Basal plate is formed of a trabecular calcified tissue.

(9) Spine is composed of dentine covered by a hard material, vitrodentine.

(10) Placoid scale contains a pulp cavity in spine.

(11) Pulp cavity contains odontoblasts dentine forming cells, blood capillaries, nerves and lymph channels.

(12) General similarity in structure of placoid scales to teeth of higher forms should be apparent. Both are considered to be remnants of bony armour of such primitive vertebrates as ostracoderms and certain placoderms.

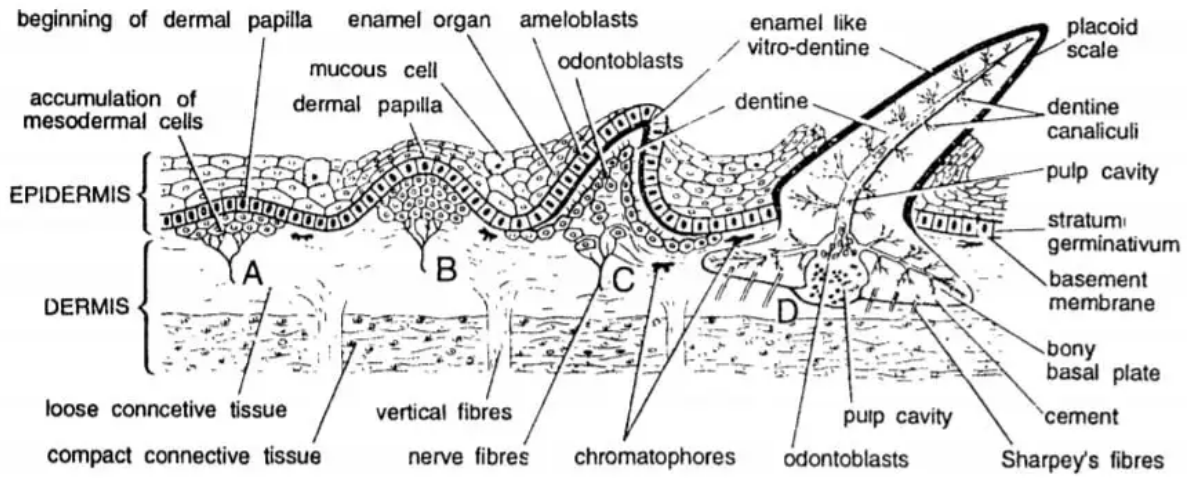


Fig.2.2 Development of Placoid Scale

Identification: Since this scale has trident spines, hence it is placoid scale of Scoliodon.

2. **Cycloid and Ctenoid Scales of Fishes:** These scales are present in teleosts or bony fishes.

Preparation: Remove a few cycloid scales from a carp or a few ctenoid scales from a koi (Anabas sp.) fish. Put them in a watch glass containing 10% KOH solution. Stir slowly with a needle till the covering epithelium dissolves. Wash thoroughly with water to remove the last trace of KOH. Make a temporary or stained permanent preparation, as required.

i. A thin, nearly rectangular plate of bone with a semicircular free border. ii. Concentric rings representing annual growth present.

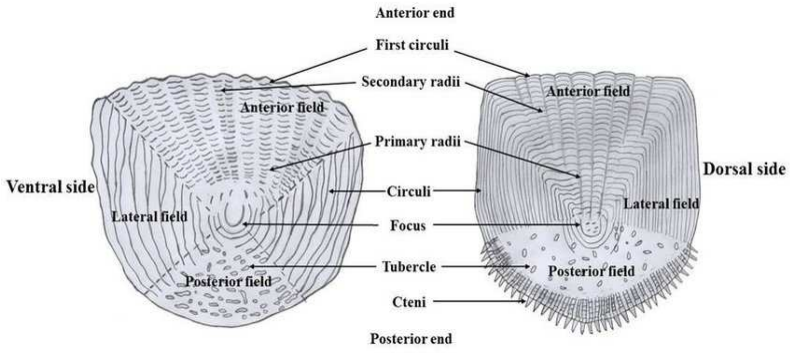


Fig.2.3

ii. Concentric rings representing annual growth present.

Cycloid scale: The free end smooth.

Ctenoid scale: The free end bears numerous short, bony spines.

AMPULLAE OF LORENZINI

For Scoliodon fish slide (Ampullae of Lorenzini) Remove a piece of skin around the snout and take out some tissue by forceps and examine it under
microscope for above ampullae. Stain in borax carmine, dehydrate, clear and mount.
Comments :
(1) Ampullae of Lorenzini are sensory and mucus-secreting structures.
(2) Each ampulla is composed of a tube and 8-9 ampullary chambers consisting of receptor and Mucus-secreting gland cells.
(3) Receptor cells are innervated by 7th cranial nerve. The tube has external opening.

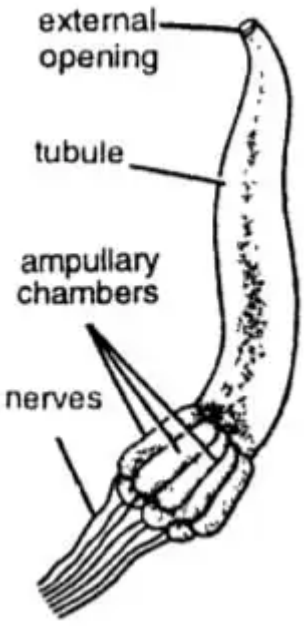


Fig.2.4 Scoliodon: - Ampullae of Lorenzini

POLYPTERUS: - GANOID SCALES

Comments:

(1) Ganoid scales are found in primitive ray-finned fishes such as Polypterus and gar pikes.
(2) Scales are covered with a hard, shiny and translucent material of mesodermal origin called as ganoin.

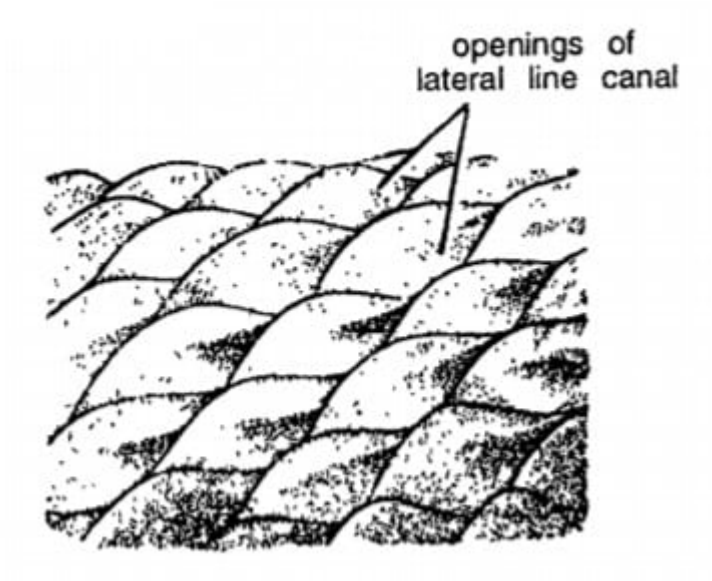


Fig 2.5

(3) Ganoid scales fit together like tiles and are arranged in diagonal rows.

(4) Scales are dermal in origin.

(5) Each scale consists of a bony base, coated by shining substance called as ganoin and openings of lateral line canal.

Identification: Since the above scale is overlapping and fitted like tiles, hence it is ganoid scale of Polypterus.

2.2 MORPHOMETRY & HISTOLOGY OF FISH BODY AND ORGANS

Fish anatomy is the study of the form or morphology of fish. It can be contrasted with fish physiology, which is the study of how the component parts of fish function together in the living fish. In practice, fish anatomy and fish physiology complement each other, the former dealing with the structure of a fish, its organs or component parts and how they are put together, such as might be observed on the dissecting table or under the microscope, and the latter dealing with how those components function together in living fish.

The anatomy of fish is often shaped by the physical characteristics of water, the medium in which fish live. Water is much denser than air, holds a relatively small amount of dissolved oxygen, and absorbs more light than air does. The body of a fish is divided into a head, trunk and tail, although the divisions between the three are not always externally visible. The skeleton, which forms the support structure inside the fish, is either made of cartilage (cartilaginous fish) or bone (bony fish). The main skeletal element is the vertebral column, composed of articulating vertebrae which are lightweight yet strong. The ribs attach to the spine and there are no limbs or limb girdles. The main external features of the fish, the fins, are composed of either bony or soft spines called rays which, with the exception of the caudal fins, have no direct connection with the spine. They are supported by the muscles which compose the main part of the trunk. The heart has two chambers and pumps the blood through the respiratory surfaces of the gills and then around the body in a single circulatory loop. The eyes are adapted for seeing underwater and have only local vision. There is an inner ear but no external or middle ear. Low frequency vibrations are detected by the lateral line system of sense organs that run along the length of the sides of fish, which responds to nearby movements and to changes in water pressure.

In many respects, fish anatomy is different from mammalian anatomy. However, it still shares the same basic body plan from which all vertebrates have evolved: a notochord, rudimentary vertebrae, and a well-defined head and tail.

Fish have a variety of different body plans. At the broadest level, their body is divided into head, trunk, and tail, although the divisions are not always externally visible. The body is often fusiform, a streamlined body plan often found in fast-moving fish. They may also be filiform (eel-shaped) or vermiform (worm-shaped). Fish are often either compressed (laterally thin) or depressed (dorso-ventrally flat).

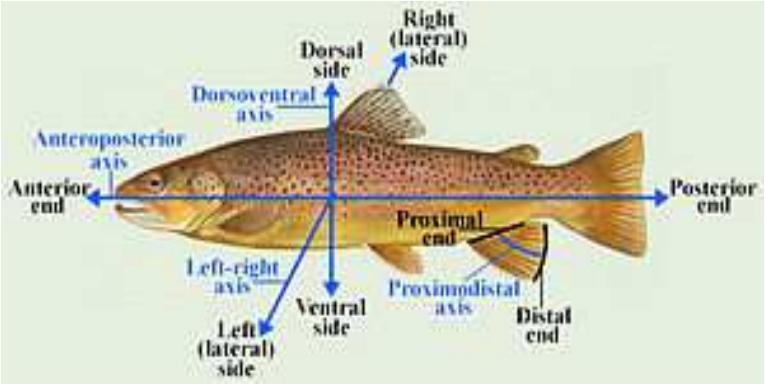


Fig.2.6 Anatomical directions and axes

2.3 STUDY OF RESPIRATORY ORGANS OF FISH

Adult fishes relied mostly on their pharyngeal gills for water breathing. Other technologies, however, are used to complement or replace gill respiration. Accessory respiratory organs are usually found in tropical freshwater fishes and are extremely seldom found in marine fishes.

Because depletion of oxygen happens during summers when the water level lowers to a significant degree, tropical freshwater and hill-stream fish can grow auxiliary respiratory organs to fulfil additional demand for oxygen. Accessory respiratory organs allow fish to thrive in oxygen-depleted water, aestivate during protracted droughts in the summer, go on terrestrial excursions, or simply satisfy increased oxygen demand. Accessory respiratory organs that can operate in an aquatic and aerial environment have been evolved in fish to solve these challenges.

As a result, the evolution of such structures is mostly adaptive in nature. Aquatic respiration is served by some accessory organs, whereas aerial respiration is served by others.

Water inhaled constantly via the mouth travels backward between the gill bars and across the gill filaments, where gases are exchanged. In teleosts and many other fishes, the gills are covered by a gill cover, but in sharks, rays, and some of the oldest prehistoric fish families, the gills are protected by skin flaps. The blood capillaries in the gill filaments are near to the gill surface, allowing them to absorb oxygen from the water and expel excess carbon dioxide.

The swim bladder, a hydrostatic (ballast) organ found in most contemporary fishes, is located in the body cavity slightly below the kidney and above the stomach and intestine. It started off as a digestive canal diverticulum. The bladder has lost its link with the digestive system in mature tetra pods, particularly acanthopterygian, a trait known as physoclistous.

Many rather primitive teleosts have kept the link (physostomous). The bladder has evolved into a lung or, at the very least, a highly vascularized supplementary respiratory organ in numerous unrelated fish species. Even in well-oxygenated water, some fish with such auxiliary organs are compulsive air breathers and would perish if refused access to the surface. Fish with a hydrostatic swim bladder may regulate the quantity of gas in the bladder to control their depth. Particular glands produce the gas, which is largely oxygen, into the bladder, making the fish more buoyant; the gas is then absorbed into the circulation by another special organ, lowering total buoyancy and allowing the fish to sink.

THE FOLLOWING ARE EXAMPLES OF FISH AUXILIARY RESPIRATORY ORGANS

Integument or Skin

Bucco – Pharyngeal Epithelium

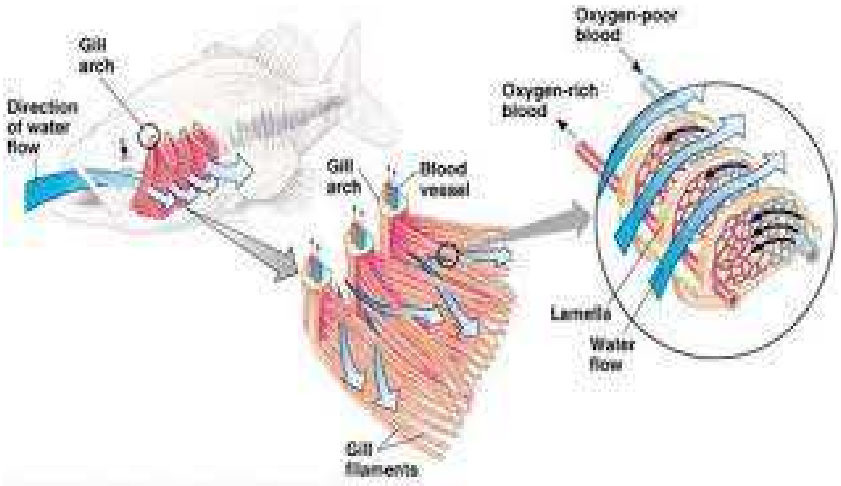
Epithelium of the Gut

Pelvic Fins Expansions

Diverticula of the Pharynx

Aerial Respiration in an Opercular Chamber

Air-Bladders



2.7

ACCESSORY RESPIRATORY ORGANS AND THEIR FUNCTIONS

The oxygen content of the auxiliary respiratory organs is greater. Fish with such respiratory organs are able to survive in water with very low oxygen concentrations. These fish come to the top of the water to gulp in air for transmission to the accessory respiratory organs in this situation. If these fish are not allowed to reach the surface, they will die of asphyxiation owing to a lack of oxygen. As a result, fishes' development of auxiliary respiratory organs is an adaptive trait.

Furthermore, it has been shown that the rate of oxygen absorption in such organs is substantially higher than the rate of carbon dioxide removal. As a result, it's only natural that the gills expel the majority of carbon dioxide. The major function of the auxiliary respiratory organs appears to be oxygen absorption.

ACCESSORY RESPIRATORY ORGAN IN FISHES

Adult fishes relied mostly on their pharyngeal gills for water breathing. Other technologies, however, are used to complement or replace gill respiration. Accessory respiratory organs refer to any extra respiratory organs that aren't gills. Accessory respiratory organs are usually found in tropical freshwater fishes and are extremely seldom found in marine fishes.

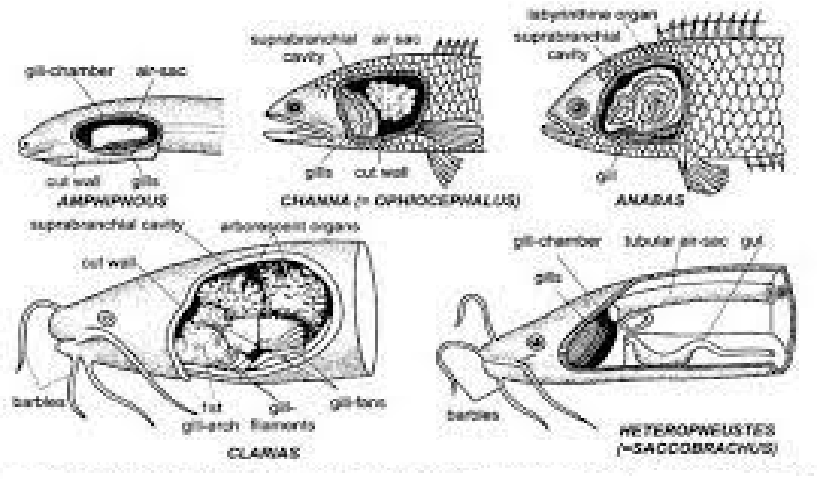


Fig.2.8 Accessory respiratory organs

Because depletion of oxygen happens during summers when the water level lowers to a significant degree, tropical freshwater and hill-stream fish can grow auxiliary respiratory organs to fulfil additional demand for oxygen. Accessory respiratory organs allow fish to thrive in oxygen-depleted water, aestivate during protracted droughts in the summer, go on terrestrial excursions, or simply satisfy increased oxygen demand.

Accessory respiratory organs that can operate in an aquatic and/or aerial environment have been evolved in fish to solve these challenges. As a result, the evolution of such structures is mostly adaptive in nature. Some auxiliary organs provide support for aquatic respiration, whereas others provide support for aerial respiration.

Accessory Respiratory Organs

Aside from the gills, all additional respiratory organs found in fish are referred to as auxiliary respiratory organs. These auxiliary respiratory organs are produced as an extra portion of the gills in fish to adapt to different conditions. These organs are most commonly encountered in tropical freshwater fish, but they are extremely rare in marine fish.

These organs are found in tropical freshwater and mountain river fish in some circumstances, especially during the summer and when the water level decreases, to fulfil the requirement for more oxygen. To defend themselves from extreme drought, some fish have been chopped to the ground for a short time.

Some fish have such a high metabolic rate they cannot be met by oxygen in the water, which has led to the development of some accessory respiratory organs for aquatic or terrestrial respiration.

Accessory respiratory organs of fish can be divided into aerial and aquatic. The teleost has 140 different types of aerial respiratory organs. When these fish spend a portion of their life on land, they rely on these organs in times of need.

2.4 STUDY OF REPRODUCTIVE ORGANS OF FISH

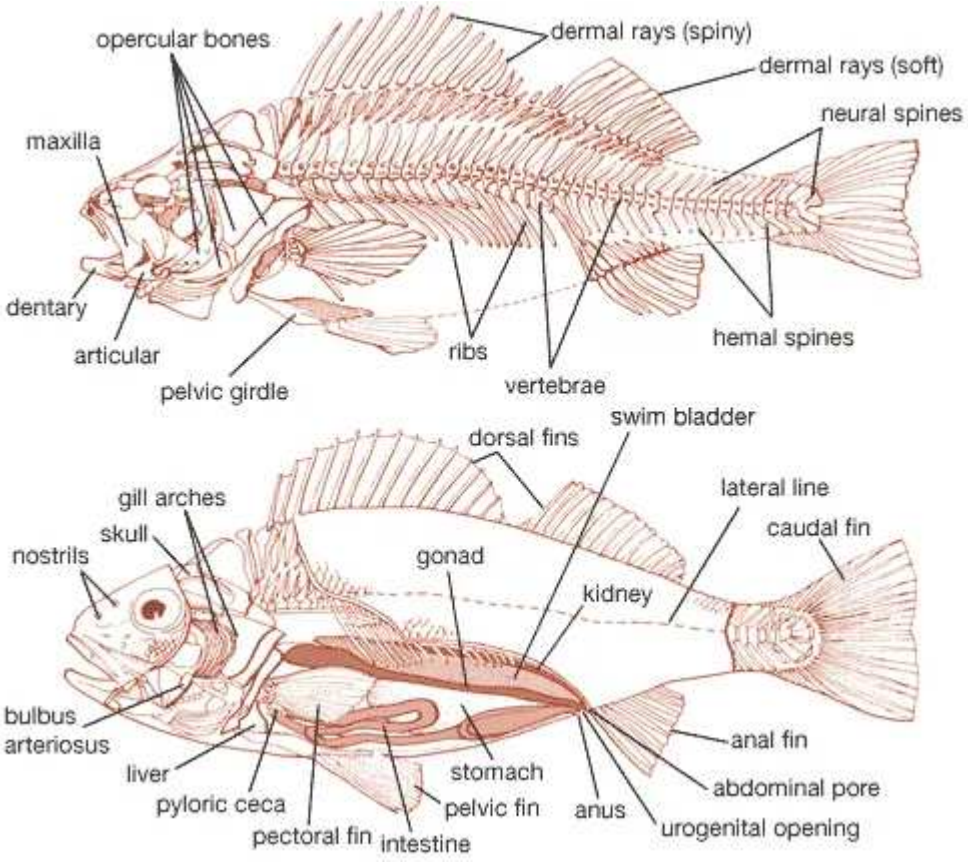
Fish reproductive organs include testes and ovaries. In most species, gonads are paired organs of similar size, which can be partially or totally fused. There may also be a range of secondary organs that increase reproductive fitness. The genital papilla is a small, fleshy tube behind the anus in some fishes, from which the sperm or eggs are released; the sex of a fish can often be determined by the shape of its papilla.

Testes

Most male fish have two testes of similar size. In the case of sharks, the testes on the right side is usually larger The primitive jawless fish have only a single testis, located in the midline of the body, although even this forms from the fusion of paired structures in the embryo.

Under a tough membranous shell, the tunica albuginea, the testis of some teleost fish, contains very fine coiled tubes called seminiferous tubules. The tubules are lined with a layer of cells

(germ cells) that from puberty into old age, develop into sperm cells (also known as spermatozoa or male gametes). The developing sperm travel through the seminiferous tubules to the rete testis located in the mediastinum testis, to the efferent ducts, and then to the epididymis where newly created sperm cells mature (see spermatogenesis). The sperm move into the vas deferens, and are eventually expelled through the urethra and out of the urethral orifice through muscular contractions.

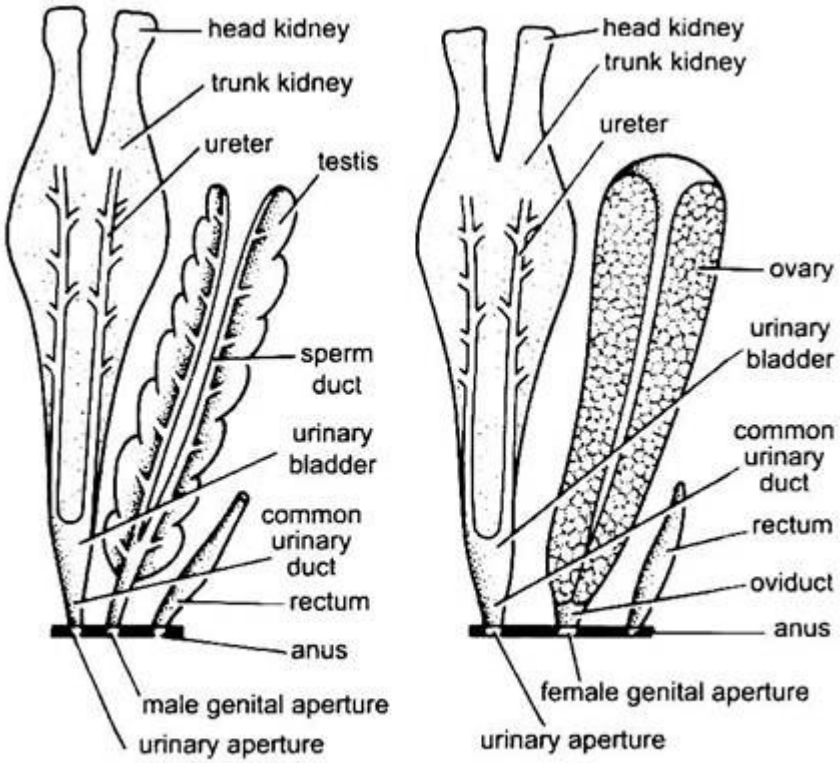


2.9 Fish reproductive organs

However, most fish do not possess seminiferous tubules. Instead, the sperm are produced in spherical structures called *sperm ampullae*. These are seasonal structures, releasing their contents during the breeding season, and then being reabsorbed by the body. Before the next breeding season, new sperm ampullae begin to form and ripen. The ampullae are otherwise essentially identical to the seminiferous tubules in higher vertebrates, including the same range of cell types.

In terms of spermatogonia distribution, the structure of teleosts testes has two types: in the most common, spermatogonia occur all along the seminiferous tubules, while in Atherinomorph fish

they are confined to the distal portion of these structures. Fish can present cystic or semi-cystic spermatogenesis in relation to the release phase of germ cells in cysts to the seminiferous tubules lumen.



Labeo. Urinogenital organs. A–Male; B–Female.

2.10

OVARIES

Many of the features found in ovaries are common to all vertebrates, including the presence of follicular cells and tunica albuginea There may be hundreds or even millions of fertile eggs present in the ovary of a fish at any given time. Fresh eggs may be developing from the germinal epithelium throughout life. Corpora lutea are found only in mammals, and in some elasmobranch fish; in other species, the remnants of the follicle are quickly resorbed by the ovary. The ovary of teleosts is often contains a hollow, lymph-filled space which opens into the oviduct, and into which the eggs are shed. Most normal female fish have two ovaries. In

some elasmobranchs, only the right ovary develops fully. In the primitive jawless fish, and some teleosts, there is only one ovary, formed by the fusion of the paired organs in the embryo.

Fig. 2.11 fish ovaries

Fish ovaries may be of three types: gymnovarian, secondary gymnovarian or cystovarian. In the first type, the oocytes are released directly into the coelomic cavity and then enter the ostium, then through the oviduct and are eliminated. Secondary gymnovarian ovaries shed ova into the coelom from which they go directly into the oviduct. In the third type, the oocytes are conveyed to the exterior through the oviduct. Gymnovaries are the primitive condition found in lungfish, sturgeon, and bowfin. Cystovaries characterize most teleosts, where the ovary lumen has continuity with the oviduct. Secondary gymnovaries are found in salmonids and a few other teleosts.

EGGS

The eggs of fish and amphibians are jellylike. Cartilagenous fish (sharks, skates, rays, chimaeras) eggs are fertilized internally and exhibit a wide variety of both internal and external embryonic development. Most fish species spawn eggs that are fertilized externally, typically with the male inseminating the eggs after the female lay them. These eggs do not have a shell and would dry out in the air. Even air-breathing amphibians lay their eggs in water, or in protective foam as with the Coast foam-nest treefrog, *Chiromantis xerampelina*.

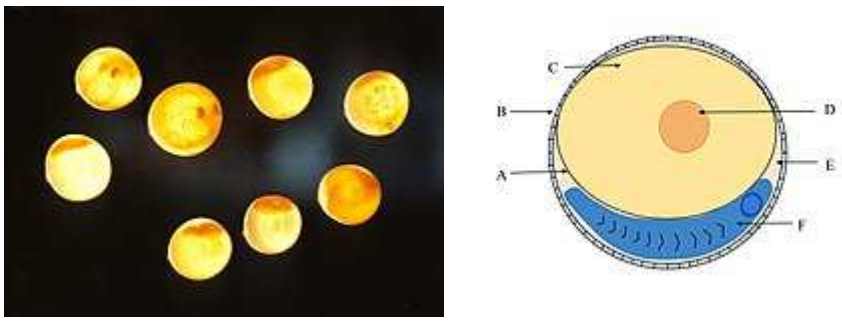


Fig. 2.12Fish Eggs

INTROMITTENT ORGANS

Male cartilaginous fishes (sharks and rays), as well as the males of some live-bearing ray finned fishes, have fins that have been modified to function as intromittent organs, reproductive appendages which allow internal fertilization. In ray finned fish they are called *gonopodiums* or *andropodiums*, and in cartilaginous fish they are called *claspers*.

Gonopodia are found on the males of some species in the Anablepidae and Poeciliidae families. They are anal fins that have been modified to function as movable intromittent organs and are used to impregnate females with milt during mating. The third, fourth and fifth rays of the male's anal fin are formed into a tube-like structure in which the sperm of the fish is ejected.[6] When ready for mating, the gonopodium becomes erect and points forward towards the female. The male shortly inserts the organ into the sex opening of the female, with hook-like adaptations that allow the fish to grip onto the female to ensure impregnation. If a female remains stationary and her partner contacts her vent with his gonopodium, she is fertilized. The sperm is preserved in the female's oviduct. This allows females to fertilize themselves at any time without further assistance from males. In some species, the gonopodium may be half the total body length. Occasionally the fin is too long to be used, as in the "lyretail" breeds of *Xiphophorus helleri*. Hormone treated females may develop gonopodia. These are useless for breeding.

2.5 STUDY OF NERVOUS & SENSORY ORGANS OF FISH

Most fish possess highly developed sense organs. Nearly all daylight fish have color vision that is at least as good as a human's (see vision in fish). Many fish also have chemoreceptors that are responsible for extraordinary senses of taste and smell. Although they have ears, many fish may not hear very well. Most fish have sensitive receptors that form the lateral line system, which detects gentle currents and vibrations, and senses the motion of nearby fish and prey. Sharks can sense frequencies in the range of 25 to 50 Hz through their lateral line.

Fish orient themselves using landmarks and may use mental maps based on multiple landmarks or symbols. Fish behavior in mazes reveals that they possess spatial memory and visual discrimination.

VISION

Vision is an important sensory system for most species of fish. Fish eyes are similar to those of terrestrial vertebrates like birds and mammals, but have a more spherical lens. Their retinas generally have both rod cells and cone cells (for scotopic and photopic vision), and most species have colour vision. Some fish can see ultraviolet and some can see polarized light. Amongst jawless fish, the lamprey has well-developed eyes, while the hagfish has only primitive eyespots. Fish vision shows adaptation to their visual environment, for example deep sea fishes have eyes suited to the dark environment.

Fish and other aquatic animals live in a different light environment than terrestrial species. Water absorbs light so that with increasing depth the amount of light available decreases quickly. The optic properties of water also lead to different wavelengths of light being absorbed to different degrees, for example light of long wavelengths (e.g. red, orange) is absorbed quite quickly compared to light of short wavelengths (blue, violet), though ultraviolet light (even shorter wavelength than blue) is absorbed quite quickly as well. Besides these universal qualities of water, different bodies of water may absorb light of different wavelengths because of salts and other chemicals in the water.

HEARING

Hearing is an important sensory system for most species of fish. For example, in the family Batrachoididae, males use their swim bladders to make advertisement calls which females use to localize males. Hearing threshold and the ability to localize sound sources are reduced underwater, in which the speed of sound is faster than in air. Underwater hearing is by bone conduction, and localization of sound appears to depend on differences in amplitude detected by bone conduction. As such, aquatic animals such as fish have a more specialized hearing apparatus that is effective underwater.

Fish can sense sound through their lateral lines and their otoliths (ears). Some fishes, such as some species of carp and herring, hear through their swim bladders, which function rather like a hearing aid.

Hearing is well-developed in carp, which have the Weberian organ, three specialized vertebral processes that transfer vibrations in the swim bladder to the inner ear.

Although it is hard to test sharks' hearing, they may have a sharp sense of hearing and can possibly hear prey many miles away. A small opening on each side of their heads (not the spiracle) leads directly into the inner ear through a thin channel. The lateral line shows a similar arrangement, and is open to the environment via a series of openings called lateral line pores. This is a reminder of the common origin of these two vibration- and sound-detecting organs that are grouped together as the acoustico-lateralis system. In bony fish and tetrapods the external opening into the inner ear has been lost.

The aquatic equivalent to smelling in air tastes in water. Many larger catfish have chemoreceptors across their entire bodies, which mean they "taste" anything they touch and "smell" any chemicals in the water. "In catfish, gustation plays a primary role in the orientation and location of food".

Salmon have a strong sense of smell. Speculation about whether odours provide homing cues, go back to the 19th century. In 1951, Hasler hypothesised that, once in vicinity of the estuary or entrance to its birth river, salmon may use chemical cues which they can smell, and which are unique to their natal stream, as a mechanism to home onto the entrance of the stream. In 1978, Hasler and his students convincingly showed that the way salmon locate their home rivers with such precision was indeed because they could recognise its characteristic smell. They further

demonstrated that the smell of their river becomes imprinted in salmon when they transform into smolts, just before they migrate out to sea. Homecoming salmon can also recognise characteristic smells in tributary streams as they move up the main river. They may also be sensitive to characteristic pheromones given off by juvenile conspecifics. There is evidence that they can "discriminate between two populations of their own species".

Sharks have keen olfactory senses, located in the short duct (which is not fused, unlike bony fish) between the anterior and posterior nasal openings, with some species able to detect as little as one part per million of blood in seawater. Sharks have the ability to determine the direction of a given scent based on the timing of scent detection in each nostril. This is similar to the method mammals use to determine the direction of sound. They are more attracted to the chemicals found in the intestines of many species, and as a result often linger near or in sewage outfalls. Some species, such as nurse sharks, have external barbels that greatly increase their ability to sense prey.

The MHC genes are a group of genes present in many animals and important for the immune system; in general, offspring from parents with differing MHC genes have a stronger immune system. Fish are able to smell some aspect of the MHC genes of potential sex partners and prefer partners with MHC genes different from their own.

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UNIT 03: FISH HABITAT ECOLOGY EXERCISE

CONTENTS

3.1 OBJECTIVES

We will learn about the Determination of CO_2,Determination of DO, Determination of pH, Determination of turbidity , Determination of total alkalinity ,Determination of hardness and the Study of pond ecosystem& Measurement of primary productivity and Microscopic study of plankton.

3.2 INTRODUCTION

The fish fauna is diverse. No other vertebrates, like birds, mammals, reptiles or amphibians, include more species than the bone fishes. They exhibit an impressive variety of adaptions to aquatic habitats. Their impact on aquatic communities and ecosystems is overwhelming. Further, fish populations provide valuable resources for humans throughout the world. This course focuses on the responses of fishes to their environments and the interactions between populations in a community. The topic of the course is essential for all students interested in fish biology, fisheries, habitat management and conservation of fish populations.

The course covers both theory and practical applications of fish ecology and behavior. It includes training in best practice of both field- and laboratory methods. The course is given at an advanced level and consists of lectures, seminars, field- and laboratory exercises and an individual project work.

3.3 DETERMINATION OF CO_2

Procedure:-

Collect 250-300 ml sample in Nessler tube carefully.

Take 100 ml sample from collected sample in a conical flask.

Add few drops of indicator if it shows pink colour then no free CO2 present in sample.

If it remain colourless then titrate it up to pink colour appearance.

A titration involves the gradual addition of a titrant solution to a solution known as an analyte. The titrant in a titration has a known concentration, whereas the analyte has an unknown concentration of some compound. In order to determine carbon dioxide levels in a solution, you can titrate it with sodium hydroxide. The carbon dioxide in the solution will react with the sodium hydroxide to form sodium bicarbonate. By using an indicator such as phenolphthalein, you can determine when all of the carbon dioxide in the water has been consumed.

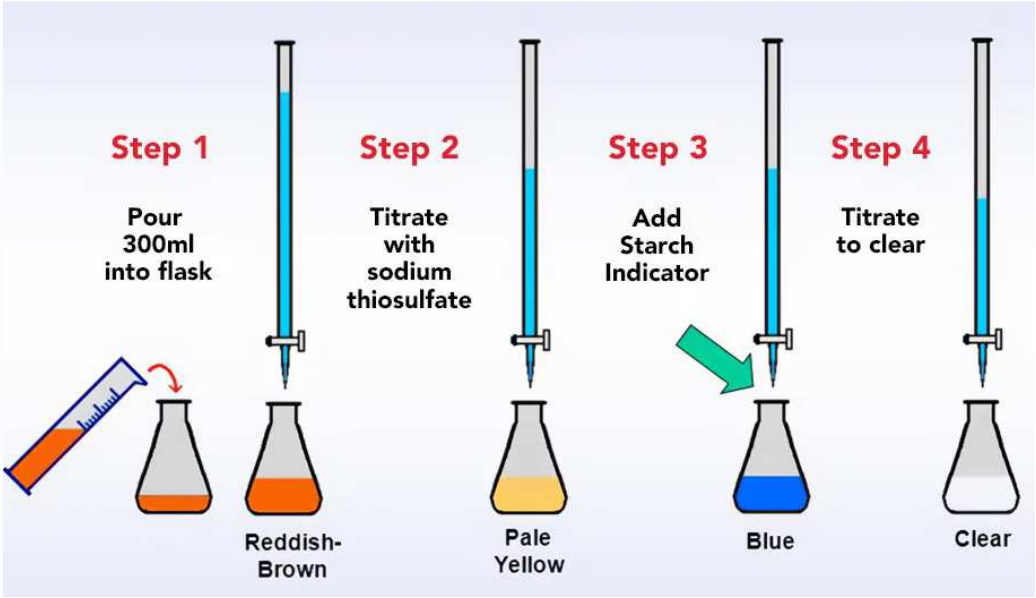

Fig. 2.1 Determination of Co_2

- Fill the graduated cylinder with a precisely known volume of the water you wish to test. Pour this into the beaker.
- Add ten drops of phenolphthalein into the water using the dropper. The phenolphthalein will turn pink if the solution is basic. Carbon dioxide lends a slight acidity to water, hence the water should remain clear when you add phenolphthalein.
- Add a couple drops of sodium hydroxide solution from the burette into the water and stir the mixture. If the solution turns light pink and stays that color, all of the carbon

dioxide has been consumed. If the solution remains clear, you must add more sodium hydroxide until the solution stays light pink.

- Multiply the number of liters of sodium hydroxide you used by the molarity of the sodium hydroxide solution. The molarity should be listed on the container. This will give you the number of moles of sodium hydroxide used in the titration. If your titration reached the endpoint and did not go beyond it, this also equals the number of moles of carbon dioxide consumed.
- Divide the number of moles of carbon dioxide by the number of liters of water you used. This will give you the molarity of the carbon dioxide in the water.

3.4 DETERMINATION OF DO

The Winkler method for dissolved oxygen (DO) is a standard titration technique to measure the oxygen content in water. Water is collected in a sample bottle with no air that could interfere with the DO reading. Fixed reagents are added to the sample to form an acidic compound.

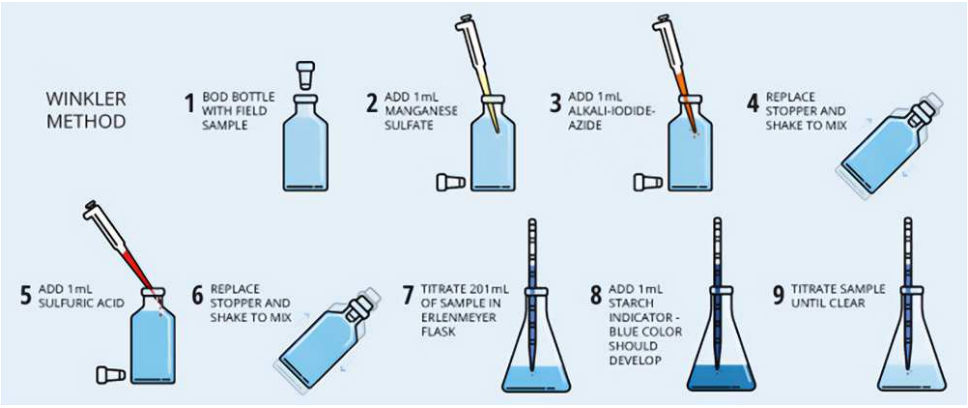

Fig.2.3 Determination of DO

The concentration of dissolved oxygen can be readily, and accurately, measured by the method originally developed by Winkler in 1888 (Ber. Deutsch Chem. Gos., 21, 2843). Dissolved oxygen can also be determined with precision using oxygen sensitive electrodes; such electrodes require frequent standardization with waters containing known concentrations of oxygen. They are particularly useful in polluted waters where oxygen concentrations may be quite high. In addition, their sensitivity can be exploited in environments with rapidly-changing oxygen

concentrations. However, electrodes are less reliable when oxygen concentrations are very low. For these reasons, the Winkler titration is often employed for accurate determination of oxygen concentrations in aqueous samples.

Collect the Water samples carefully in 250 ml glass stoppered sampling bottles within the water body to exclude air bubbles. 1.0 ml each of manganous sulphate and alkaline iodide reagents were added by means of 1 ml pipette, that will immersed to the bottom of the bottle and the pipette will be slowly drawn out after addition. The stopper was replaced and the bottle will be inverted 3-4 times for thorough mixing. The resultant flocculent wills dissolved by adding 1 ml concentrated H2SO4, mixed and dissolved by gentle inversion. 50 ml of this solution was then transferred to an Erlenmeyer flask and 0.025 N Na2S2O3 solution was added drop by drop till the colour turned to pale straw. Few drops of starch solution were added and titration continued to reach the end point, i.e. disappearance of the blue colour.

Calculation: Dissolved oxygen (mg/l) = ml of 0.025 N Na2S2O3 used × 4

3.5 DETERMINATION OF pH

pH is measured using pH meter, which comprises a detecting unit consisting of a glass electrode, reference electrode, usually a calomel electrode connected by KCl Bridge to the pH sensitive glass electrode and an indicating unit which indicates the pH corresponding to the electromotive force is then detected.

The term “pH” refers to the measurement of hydrogen ion activity in the solution.Since the direct measurement of the pH is very difficult, specific electrodes are needed for quick and accurate pH determination. pH is measured on a scale of 0 to14, with lower values indicating high H+ (more acidic) and higher values indicating low H+ ion activity (less acidic). A pH of 7 is considered as neutral. Every whole unit in pH represents a ten-fold increase in or decrease in hydrogen ion concentration. Most natural waters possess the pH values ranging from 5.0 to 8.5. Rain water have a pH value of 5.4 to 6.0 which then reacts with the soils and minerals causing the reduction in H+ ion concentration and thus the water may

become alkaline with a pH 0f 8.0-8.5. More acid water (pH<5) and more alkaline (pH >9) and other immediate changes in the hydrogen ion concentration (pH) suggest that the quality of the water is adversely affected due to the introduction of some toxic contaminants in water

bodies.pH is measured using pH meter, which comprises a detecting unit consisting of a glass electrode, reference electrode, usually a calomel electrode connected by KCl Bridge to the pH sensitive glass electrode and an indicating unit which indicates the pH corresponding to the electromotive force is then detected. Before measurement, pH meter should be calibrated by using at least two buffers.

EQUIPMENT REQUIRED:-

1. pH meter
2. pH electrode filled with KCL solution
3. Buffer solutions of pH 4 and pH 7 4. Clean beakers
5. Tissue papers
6. Distilled water
7. Thermometer

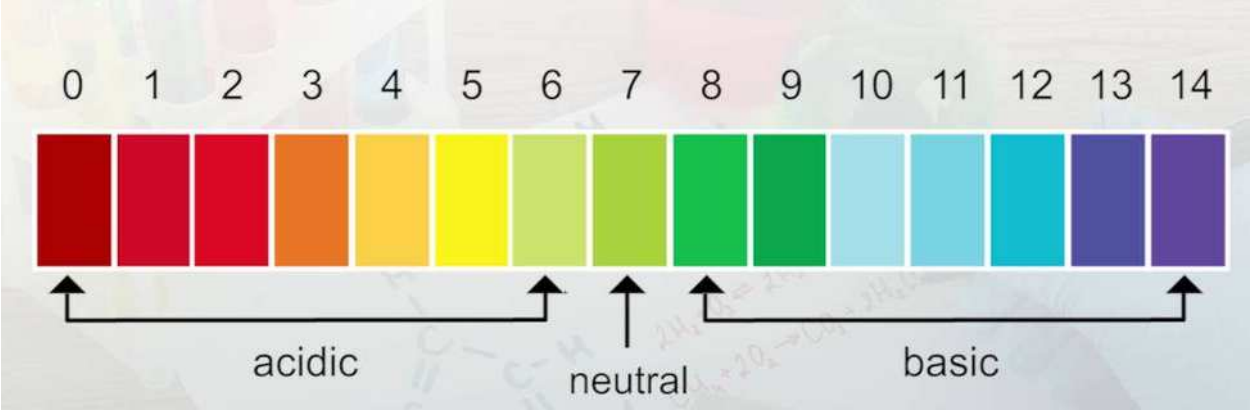

Fig.2.4 pH Scale

Procedure:- Plug in the pH meter to power source and let it warm up for 5 to 10 minutes

- Wash the glass electrode with distilled water and clean slowly with a soft tissue.
- Note the temperature of water and set the same on the pH meter
- Place the electrode in pH 7 buffer solution and set the value of 7 on the pH meter turning the Calibrate knob on the meter.
- Take out the electrode, wash with DW and clean.
- Dip the electrode in the pH 4 buffer solution. Adjust the value on the pH readout meter by the Slope switch. Repeat with pH 7 and pH4 buffers till a correct and stable reading is displaced.

- While moving and cleaning the electrode, put the selector switch on standby mode. Turn to pH mode for recording the pH.
- Now place the electrode in the water sample whose pH is to be determined.
- You can take a number of simultaneous readings for different samples until the power is on.

3.6 DETERMINATION OF TURBIDITY

Turbidity is commonly measured in Nephelometric Turbidity Units (NTU). The nephelometric method compares how light is scattered in a water sample against the amount of light scattered in a reference solution. An electronic hand-held meter is often used to measure turbidity.

Principle

Turbidity is dependent on the comparison of the intensity of light scattered by the sample under characterized conditions with the power of the light scattered by a standard reference suspension under similar conditions.The turbidity of the sample is thus estimated from the amount of light scattered by the sample taking a reference with standard turbidity suspension. The higher the power of scattered light the more the turbidity.For the primary standard reference, suspension polymer is used. The unit of turbidity is expressed on NTU (Nephelometric Turbidity Unit).

Reagents:

1. Turbidity-free water:

Generally, Distilled water is considered turbidity-free water for any test.

2. Stock turbidity solutions

Solution 1:- For making a solution dissolve 1.0 grams of hydrazine sulfate $(NH_2)_2.H_2So_4$ in distilled water and dilute it to 100 ml in a makeup flask.

Solution 2:- For the 2^{nd} Solution dissolve 10.0 grams hexamethylenetetramine $(CH_2)_6N_4$ in distilled water and dilute it to 100ml.

Solution 3:- Now, Mix solutions 1 and 2 in a 100ml flask each 5ml, and left it to stand for at least 24 hs., then dilute it to 100ml and mix thoroughly. The turbidity of this solution is 400 NTU.

Standard Turbidity Solution:- For making a standard turbidity solution take 10.0ml of solution 3 in 100ml to make up the flask and dilute it to 100ml. with turbid-free water. The turbidity of this suspension is 40 NTU.

Determination of turbidity of sample water

1. Turbidity less than 40 units

It is essential to allow samples to come to room temperature before analysis. Mix the sample to thoroughly disperse the solids.

Wait until air bubbles disappear then pour the sample into the turbidity meter tube. Now, read the turbidity value in NTU directly from the instrument display or from the appropriate calibration curve.

2. Turbidity exceeding 40 units

In case of turbidity of the sample exceeds 40 units, and then add one or more same volume of distilled water or turbidity-free water in the turbid sample until the turbidity falls below 40 units. The turbidity of the test sample is calculated from the turbidity of the diluted sample and the dilution factor.

For example, if 5 same volumes of distilled water were added to the turbid sample, and the diluted sample showed turbidity of 30 units, then the turbidity of the original sample was 180 units.

$$\text{Nephelometric Turbidity Units (NTU)} = A(B + C) / C$$

Where,

A = Turbidity of the diluted sample, B = Volume of a diluted sample (ml)

C = Sample volume taken for dilution.

Observations:

For undiluted sample	For diluted sample
Digital readout =	Vol. of sample (C) =
	Vol. of dilution water (B) =
	Digital readout (A) =

CALCULATIONS:

For undiluted sample	For diluted sample
Turbidity of sample (NTU) =	Turbidity in NTU = A(B + C) /C

3.7 DETERMINATION OF TOTAL ALKALINITY

Total alkalinity is measured by collecting a water sample, and measuring the amount of acid needed to bring the sample to a pH of 4.2. At this pH all the alkaline compounds in the sample are "used up." The result is reported as milligrams per liter (mg/l) of calcium carbonate. and pH less than 5.0.

Alkalinity is a measure of a river's "buffering capacity," or its ability to neutralize acids. Alkaline compounds in the water such as bicarbonates (baking soda is one type), carbonates, and hydroxides remove H+ ions and lower the acidity of the water (which means increased pH). They do this usually by combining with the H+ ions to make new compounds. Without this acid neutralizing capacity, any acid added to a river would cause an immediate change in the pH.

Measuring alkalinity is important to determining a river's ability to neutralize acidic pollution (as measured by pH) from rainfall or snowmelt. It's one of the best measures of the sensitivity of the river to acid inputs. Alkalinity comes from rocks and soils, salts, certain plant activities, and certain industrial wastewater discharges. **Alkalinity** is a measure of a river's "buffering capacity," or its ability to neutralize acids. Alkaline compounds in the water such as bicarbonates (baking soda is one type), carbonates, and hydroxides remove H+ ions and lower the acidity of the water (which means increased pH). They do this usually by combining with the H+ ions to make new compounds. Without this acid neutralizing capacity, any acid added to a river would cause an immediate change in the pH. Measuring alkalinity is important to determining a river's ability to neutralize acidic pollution (as measured by pH) from rainfall or snowmelt. It's one of the best measures of the sensitivity of the river to acid inputs. Alkalinity comes from rocks and soils, salts, certain plant activities, and certain industrial wastewater discharges.

Alkalinity Measurement

Equipment required:

- pH-Meter
- Refillable Electrode
- Buffers (4.01 and 7.00)
- Deionized or distilled water
- 150ml Glass Beaker
- Magnetic Stirrer
- Stir Bar
- 100ml Graduated Cylinder
- Digital Titrator
- 0.16N Sulfuric Acid Cartridge

After placing the sulfuric acid cartridge in position in the Hach Digital Titrator, be sure to advance the plunger manually until titrant is forced out of the delivery tip. Do this as you would a hypodermic syringe, with the delivery tip up to remove bubbles. Get all the bubbles out! Then advance the plunger using the delivery knob on the end of the titrator until you are sure that the delivery tip is filled with solution. Check for leaks where the tip connects to the cartridge. Rinse

the tip WELL with distilled water or sample; this is important because the titrant is concentrated and a little bit goes a long way. Reset the counter to zero and you are ready to titrate.

After completing a titration and recording the digits of titrant used, rinse the delivery tip with distilled water or the next sample, reset the counter (THIS IS EASILY FORGOTTEN WHEN BUSY), and you are immediately ready for the next sample.

Titrations go better if the delivery tip is positioned under the surface of the solution being titrated. For one or two samples, the titrator can be held in the hand, however, it is easier to mount the titrator on a ring stand using a clamp. Try to keep the titrator vertical through all titrations; putting the titrator horizontally on the bench between titrations may introduce bubbles in the tip.

The acid cartridges provided are 0.16N sulfuric acid. Our waters are typically quite low in alkalinity, so we use a special double end-point alkalinity procedure to accurately measure alkalinity below 20 mg L-1.

After reading and recording the pH as described above, titrate

with the digital titrator and sulfuric acid cartridge to pH 4.5; record titrant used to this point as A. Continue the titration to pH 4.2. Record the titrant used to this point as B. If the initial pH is less than 4.5, record the initial pH value. Titrate until the pH is 0.3 units below the starting point. Enter the digits of titrant used as B; A = 0. Write down the pH reading where you stopped (as an accuracy check). We will use computers to calculate the alkalinity, but you may do your own calculations using the formulas below. The examples will help to clarify what can be somewhat confusing formulas.

A = digits used to pH 4.5

B = digits used to pH 4.2 or 0.3 pH units below initial value (total titrant including A)

Double end-point alkalinity= (2A - B) x 0.1

3.8 DETERMINATION OF HARDNESS

The estimation of hardness is based on complexometric titration. Hardness of water is determined by titrating with a standard solution of ethylene diamine tetra acetic acid (EDTA) which is a complexing agent. Since EDTA is insoluble in water, the disodium salt of EDTA is taken for this experiment.

Hardness in water is due to the presence of dissolved salts of calcium and magnesium. It is unfit for drinking, bathing, washing and it also forms scales in boilers. Hence it is necessary to estimate the amount of hardness producing substances present in the water sample. Once it is estimated, the amount of chemicals required for the treatment of water can be calculated. The estimation of hardness is based on complexometric titration. Hardness of water is determined by titrating with a standard solution of ethylene diamine tetra acetic acid (EDTA) which is a complexing agent. Since EDTA is insoluble in water, the disodium salt of EDTA is taken for this experiment. EDTA can form four or six coordination bonds with a metal ion. Two type of hardness is present in water first is temporary hardness and second is permanent hardness. Temporary hardness is due to the presence of bicarbonates of calcium and magnesium ions. It can be easily removed by boiling. Permanent hardness is due to the presence of chlorides and sulphates of calcium and magnesium ions. This type of hardness cannot be removed by boiling. Requirements: Water sample Burette 25-30ml Glass funnel Pipette 1ml Flask Dropper Measuring cylinder

Reagents:

EDTA, Eriochrome Black-T, NH2CL, Ammonia Buffer, Magnesium Carbonate, 90% ethyl alcohol, Distilled water.

Reagent preparation: 1. EDTA solution: 4gm EDTA and 0.1gm magnesium bicarbonate dissolve in 800 ml distilled water.

2. Eriochrome Black-T: 0.4gm Ericrome Black T, 4.5 gm hydroxylmine hydrochloride add in 100ml 95% ethyl alcohol.

3. Ammonia Buffer: Stock A: 16.9gm of NH4CL in 143ml of conc. NH4OH, Stock B: 1.25gm magnesium salt of EDTA dissolves in 50 ml distilled water. Mix both stock solutions and dilute to 250ml with DDW. Dilute 10ml of the solution to 100ml with DDW.

Procedure:

1. The burette is filled with standard EDTA solution to the zero level.

2. Take 50ml sample water in flask. If sample having high Calcium content then take smaller volume and dilute to 50ml.

3. Add 1ml Ammonia buffer.

4. Add 5 to 6 drop of Ericrome black – T indicator. The solution turns into wine red colour.

5. Note the initial reading.

6. Titrate the content against EDTA solution. At the end point colour change from wine red to blue colour.

7. Note the final reading and record it. Repeat the process till we get concordant value.

8. Take 50ml sample in another flask and boiled it. (Add distilled water to get final volume of water.)

9. Repeat step 3-7. Calculations:

Total hardness of water mg/L (CaCO3 Scale) = ml of EDTA used (unboiled)

*103 /ml of sample Permanent hardness of water mg/L (CaCO3 Scale) = ml of EDTA used (boiled)

*103 /ml of sample Temporary hardness of water mg/L (CaCO3 Scale) = Total hardness of water - Permanent hardness of water Observation: The colour of soluble distilled water and R.O water instantly changed into blue while tap water and pond water turned wine red when Ericrome black T was added and therefore after turned blue when titrated against EDTA solution.

3.9 STUDY OF POND ECOSYSTEM

Pond Ecosystem:

A biological community made up of various species of creatures is referred to as an ecosystem. To adapt to changing environmental conditions, these organisms communicate with one another. Around us, there are various ecological types. The existence of nearby environmental factors may also be used to define an ecosystem. Pond ecosystems, forest ecosystems, ocean ecosystems, and others are some of the best examples of ecosystems. The pond serves as an illustration of an ecosystem created in a region where water overflows and contains aquatic

plants and animals. An ecosystem is created when the many kinds of plants and animals in a pond ecosystem interact with one another and their surroundings.

Pond Ecosystem: Definition

Pond ecology is a type of freshwater ecosystem that can be temporary or permanently made up o f a range of aquatic organisms interacting with one another and the aquatic environment around them. The longer amount of time that the water is stagnant makes the pond environment a lentic ecosystem.

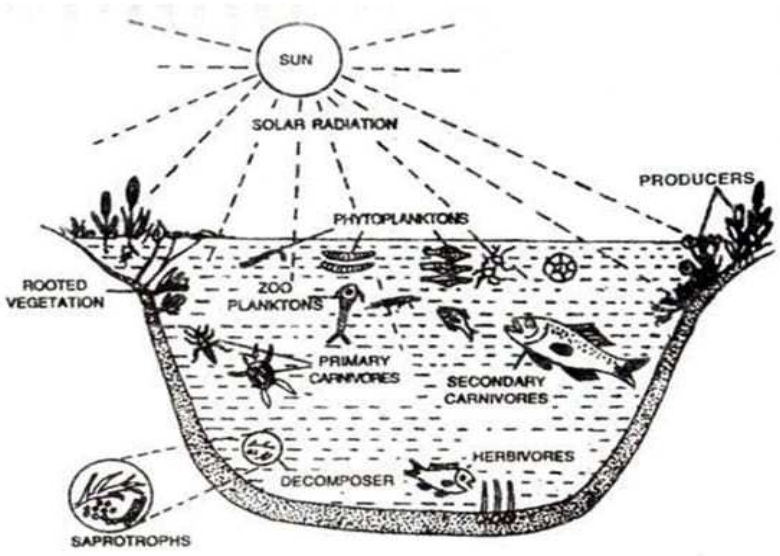

Fig2.4.: Pond Ecosystem

Types of Pond Ecosystem

There are the following types of pond ecosystems:

- **Garden pond ecosystems:**

These are artificial ponds that were created by humans and contain decorative plant and animal species imported from all over the world.

- **Salt pond environments:**

These ecosystems, which contain brackish water, are generated naturally by the sea. These result from water logging. These can also be found in rock pools, which are rocky regions on the beach. Because it contains brackish water, it can support marine life.

- **Freshwater pond ecosystems :**

Freshwater pond ecosystems are created naturally as a result of rainfall or soil saturation brought on by on-going rain. They may also develop as a result of river water flowing into a significant depression. Freshwater fish, amphibians, crabs, and many other species of wildlife all call these habitats home.

- **Venereal pond ecosystems:**

These are temporary ponds that occur during periods of heavy rain as a result of the buildup of water in the ground depressions. They frequently become desert terrain as the seasons change.

- **Mountain pond ecosystems:**

Ponds that have naturally formed might be found in mountainous areas. These are the result of rock movement and snow melting. They provide habitat for rare or threatened aquatic species.

Characteristics of Pond Ecosystem:

The following are the main characteristics of the pond ecosystem:

1. The pond environment is surrounded by either natural or man-made barriers; the water in the pond is stagnant.
2. The littoral zone, limnetic zone, profundal zone, and benthic zone are the three separate zones that make up the pond ecosystem.
3. The biotic elements of the pond ecosystem are located at various levels, which prevents them from competing with one another for life.
4. Scavengers and decomposers occupy the bottom level, and fish occupy the middle level.
5. Pond ecosystems demonstrate a wide range of variability in their size. The plants enclose the pond's edges and offer protection to little creatures and insects.
6. Pond ecosystems show a wide range of variety in their size.

Stratification in the Pond Ecosystem

The following zones are found in the pond ecosystem, and they are determined by various characteristics including proximity to the shore, light penetration, water depth, plant and animal species, etc.

- **Littoral zone** : The zone closest to the shore is known as the **littoral zone**. Shallow water is present, and light can easily enter. It is home to rooted plant species. Reeds, crawfish, snails, insects, etc. are examples of different animal species.
- **Limnetic zone**: The open water of a pond in which light can effectively penetrate is referred to as the **limnetic zone**. Phytoplankton dominates this region. Small fish and insects are the two predominant animal species.
- **Profundal zone**: An area of a pond below the limnetic zone is referred to as a profound zone since it is completely dark. It is home to certain turtles and amphibians.
- **Benthic zone**: The bottom zone of a pond is benthic and is occupied by a community of decomposers. The decomposers are called benthos.

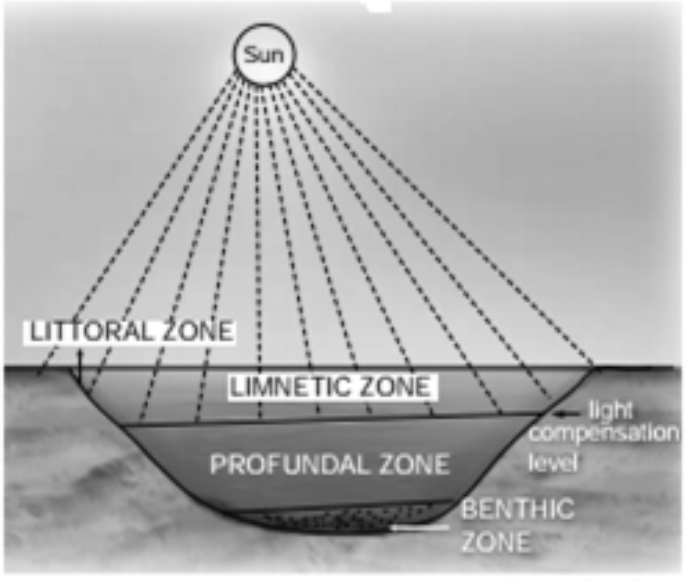

Fig 2.5: Stratification of the Pond Ecosystem

Abiotic Components of the Pond Ecosystem

The non-living elements of an ecosystem known as abiotic components are crucial for the survival of aquatic life. The following are the pond ecosystem's primary abiotic elements:

1. **Light:** Light is a key abiotic element needed for phytoplankton to engage in photosynthetic activity. Light penetrates most deeply in the littoral zone and least deeply in the profound zone.
2. **Temperature:** The progressive reduction in light penetration causes the temperature of the water to gradually drop as the depth of the pond increases.
3. **Dissolved oxygen:** As you move from the pond's surface to its depth, the amount of dissolved oxygen steadily drops. It is highest in the shallow water.

Biotic Components of the Pond Ecosystem

Living things make up biological components. The following list of living organisms that make up the pond ecosystem can be discussed:

1. **Producers:** These comprise a variety of rooted, submerged, emergent, floating, and algal species. *Spirogyra* is the most prevalent type of filamentous algae found in ponds. Other algae in the pond include *Mougeotia* and *Zygnema.* In the pond ecosystem, green plants like *Azolla, Hydrilla, Pistia, Wolffia, Lemna, Eichhornia, Nymphaea, Potamogeton, Jussiaea, etc*. can be found.
2. **Primary consumers:** The principal primary consumers are a sizable population of zooplanktons. The main consumers typically found in the pond are small herbivores like snails, insects, small fish, tadpoles, and larvae of aquatic creatures. Large animal species like frogs, large fish, water snakes, crabs, etc. are examples of secondary consumers. Mammals such as water shrews and water voles, as well as herons, ducks, kingfishers, and other birds, may be among the highest order of consumers.
3. **Decomposers:** These are the various bacterial and fungal species that consume the dead and decomposing components of aquatic species.

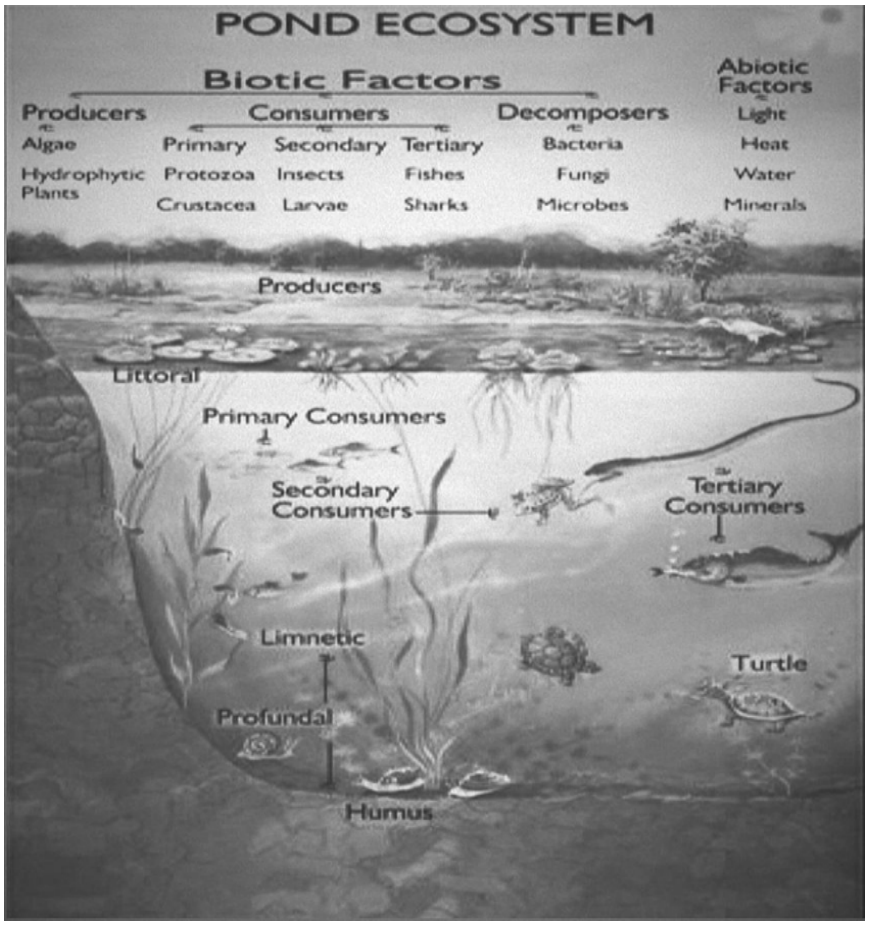

Fig.2.6: Biotic and Abiotic Factors of the Pond Ecosystem

Food Chain in the Pond Ecosystem

The food chain is a group of animals in which each one consumes the lower member before being consumed by the species above it. Algae and phytoplankton act as producers, converting solar energy into chemical energy. Zooplankton is consuming phytoplankton (primary consumers). Small pond creatures that eat zooplankton continue the food chain's progression. Large pond species consume little pond species. Some bacteria and fungi are known as decomposers because they consume dead and rotting animal species' components. Decomposers break down organic materials (dead plants and animals) into their inorganic constituents, which are then used again by producers. This maintains a constant flow of energy.

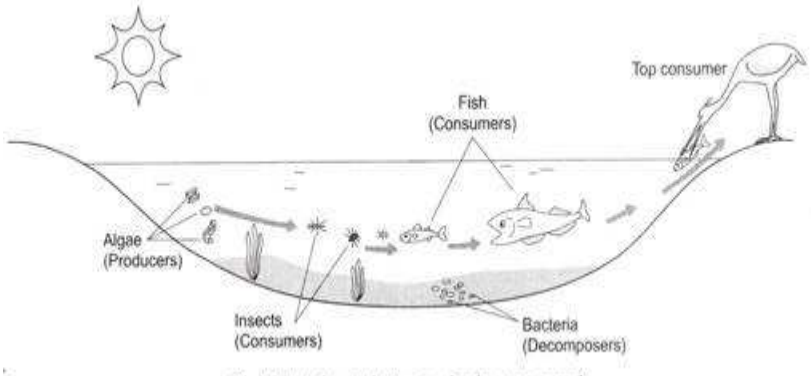

Fig. 2.7: Food Chain in the Pond Ecosystem

Importance of Pond Ecosystem

The following points can be made regarding the significance of pond ecosystems:

- Some aquatic plants contribute to better water quality by removing heavy metals and pollutants from the water.
- Shoreline plants absorb nitrogen and phosphorus, which helps to prevent algal blooms and maintains the pond's oxygen level.
- The pond ecosystem is one of the sites for the conservation of biodiversity as different types of plants and consumers occupy different strata in the pond and live together by interacting with each other. In addition, aquatic plants absorb animal wastes to reduce the nutrient availability for plants and thereby prevent the growth of algae. Mountainous ponds protect the endangered species.
- Pond ecosystems add to the beauty of nature by supporting a range of decorative flowering plants, as well as acting as a supply of water for species that do not dwell in the pond.
- The distribution of animal species within the pond is governed by stratification in the pond ecosystem. It somewhat lessens competition between the species.
- Pond ecology is best defined as the relationship between the life in your pond and its surroundings.
- Algae and aquatic plants will flourish in a shallow, nutrient-rich pond that is exposed to sunshine and has little water running through it. Due to the low oxygen levels, there may not be much animal life there.

- A newly constructed, deep, spring-fed pond, on the other hand, might not have any life in it at all due to the cold temperatures and scarcity of food.
- Every pond ages. A pond's initial components are mainly water, few nutrients, and not much aquatic life. The pond builds up nutrients over time. The term "**eutrophication**" refers to this enrichment process. Aquatic life grows as a result of the supply of nutrients. These organisms are alive, develop, and pass away.
- Their remnants decompose in the pond, releasing the nutrients needed to grow them back into the water to continue the cycle. However, eventually there will be a buildup of material that is resistant to degradation, and the pond will overflow. It will eventually turn into a bog and resemble dry terrain.

Returning to dry land can take a decade or it can take generations. It is your responsibility as the pond owner to delay the process as much as you can. The guidelines you can use are described below.

3.9.1 MEASUREMENT OF PRIMARY PRODUCTIVITY

Primary productivity is usually determined by measuring the uptake of carbon dioxide or the output of oxygen. Production rates are usually expressed as grams of organic carbon per unit area per unit time.

primary productivity, in ecology, the rate at which energy is converted to organic substances by photosynthetic producers (photoautotrophs), which obtain energy and nutrients by harnessing sunlight, and chemosynthetic producers (chemoautotrophs), which obtain chemical energy through oxidation. Nearly all of Earth’s primary productivity is generated by photoautotrophs.

Calculating primary productivity

The total amount of biological productivity in a region or ecosystem is called the gross primary productivity. A certain amount of organic material is used to sustain the life of producers (or autotrophs) in a food chain, and what remains is the net primary productivity, which can be used by consumers (or heterotrophs, which are made up of herbivores and carnivores in each environment). Primary productivity is usually determined by measuring the uptake of carbon dioxide or the output of oxygen. Production rates are usually expressed as grams of organic carbon per unit area per unit time.

TYPES OF PRIMARY PRODUCERS

In marine environments, the two principal categories of producers are pelagic phytoplankton, which float freely in the ocean, and benthic algae, which live at or near the ocean's floor. In terrestrial environments, primary productivity is generated by trees and other land plants (including planted crops). Most primary producers require nitrogen and phosphorus—which are available as dissolved nutrients in the soil, lakes, and rivers and in the oceans as nitrate, nitrite, ammonia, and phosphorus. The abundances of these molecules and the intensity and quality of light exert a major influence on rates of production.

The annual productivity of the entire ocean is estimated to be approximately 50 × 1015 grams (50 × 109 metric tons) of carbon per year, which is about half of the global total. Most primary productivity in the oceans is carried out by free-floating phytoplankton in the open ocean rather than by bottom-dwelling (benthic) plants, with chemoautotrophs contributing smaller amounts as producers in deep-sea-vent habitats. Benthic plants grow only on the fringe of the world's oceans and are estimated to produce only 5 to 10 percent of the total marine plant material in a year.

3.9.2 MICROSCOPIC STUDY OF PLANKTON

Plankton are the mostly microscopic plants and animals that drift in the currents. Plant plankton is called "phytoplankton," while animal plankton is called "zooplankton." Plankton forms the basis of life in the sea. Plankton samples may be obtained in several different ways.

PHYTOPLANKTON

Phytoplankton may be described as free-floating microscopic plants. This is because they are tiny organisms that are capable of using carbon dioxide and sunlight to produce their own food. Like other plans that grow on land, phytoplankton has chlorophyll in their cells used for photosynthesis. This allows them to trap sunlight, which is then converted to chemical energy in the presence of carbon dioxide.

TYPES OF PHYTOPLANKTON

- Diatoms (e.g., Bacillariophyta)
- Cyanobacteria (e.g., Synechococcales, Spirulina)
- Dinoflagellates (E.g., Noctilucales)

Phytoplankton are very diverse and exist as both prokaryotic (e.g. cyanobacteria) and eukaryotic (algae) forms. This is a big advantage given that phytoplanktons are the primary producers in aquatic bodies.

However, some phytoplankton like sapromixotrophs and phagomixotrophs are classified as mixotrophs because they are not only capable of producing their own food through photosynthesis, but also obtain nutrients from organic material present in their environment.

As the primary producer, phytoplankton plays a very important role in the aquatic food web. By feeding other plankton like zooplankton as well as other small fish, phytoplankton make it possible to feed other bigger aquatic organism like whales. Therefore, in the absence of phytoplankton, other higher aquatic organisms would be highly affected and even die off.

Apart from their importance in the aquatic food web, phytoplankton also plays a very important role in the carbon cycle. As earlier mentioned, phytoplankton use carbon dioxide for photosynthesis in order to produce food (chemical energy). In the process, some of the carbon is stored in phytoplankton when they die and settle at the bottom of the sea.

Any change in the growth and amount of these organisms therefore has a direct impact on global atmospheric carbon-dioxide gas and thus on the temperature. Because they carry some of the carbon to the bottom of the ocean when they die, phytoplankton also contributes to the formation of oil. While phytoplankton is important, they can cause diseases and even kill both marine life and people. Some of the species have been shown to produce dangerous bio-toxins that result in red tides and algal blooms. When consumed, these toxins can cause serious illnesses and even cause death.

ZOOPLANKTON

Zooplankton is a variety of minuscule animals with limited swimming abilities. As such, they are incapable of swimming against the current and thus go where the current takes them.Unlike phytoplankton that are capable of manufacturing their own food, zooplankton survive by feeding on phytoplankton since they lack chlorophyll. The different types of zooplankton can be grouped on the basis of size and how they develop.

Size - Based on size, zooplanktons are classified as:-

- **Picoplankton** - Picoplankton are the smallest zooplankton that measuring less than 2 micrometers. Examples of picoplankton include Synechococcus, picoeukaryotes and Prochlorococcus.
- **Nanoplankton** - Nanoplankton may range between 2 and 20 micrometers and include such zooplankton as Pyrrophyta, Xanthophyta and Chrysophyta.
- **Micro plankton** - Micro plankton measure between 20 and 200 micrometers and include certain small copepods
- **Mesoplankton** - Mesoplankton range between 0.2 to 20 mm in size and include euphausids and some larval fish organisms
- **Macro plankton** - Measuring between 20 and 200 mm, examples of macro plankton include larger crustaceans and jellyfish
- **Mega plankton** - Mega plankton are over 200 mm in size and includes larger jellyfish.

IMPORTANCE (IN AQUATIC ENVIRONMENT)

Like phytoplankton, zooplankton also plays an important role in the aquatic food web. By feeding primarily on phytoplankton (algae), zooplankton prevents algae from growing out of control.

As already mentioned, changes in the growth of phytoplankton has a direct impact on the global carbon cycle. Therefore, by consuming phytoplankton, zooplankton also plays an important role in the regulation process.

Zooplanktons are also important food sources for various organisms including planktivorous fish. As such, they play an important role in aquatic food web given that their absence would affect the rest of organisms above them in the food chain.

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UNIT 04: FISH PHYSIOLOGY AND BIOCHEMISTRY EXERCISES

CONTENTS

4.1 OBJECTIVES

We will learn about the Material and Methods for estimation/counting of Protein, Lipids and Hematological analysis of fish blood and Hemoglobin in fish blood. Study of WBC/ RBC/DLC in fish blood Results.

4.2 INTRODUCTION

Fish physiology is the scientific study of how the component parts of fish function together in the living fish. It can be contrasted with fish anatomy, which is the study of the form or morphology of fishes. In practice, fish anatomy and physiology complement each other, the former dealing with the structure of a fish, its organs or component parts and how they are put together, such as might be observed on the dissecting table or under the microscope, and the later dealing with how those components function together in the living fish. For this, at first we need to know about their intestinal morphology.

4.3 MATERIAL AND METHODS FOR ESTIMATION/COUNTING

4.3.1 PROTEIN

PROTEIN DETERMINATION BY KJELDAHL METHOD:

The crude protein was estimated by micro kjeldahl technique. 2 g of the dried sample was taken in the long necked 100 ml digestion flask. To this 2.0-5.0 g of the digestion mixture (K2SO4 and CuSO4 in the ratio of 9:1) and 10-30 ml concentrated H2SO4 were added. The mixture was gently heated till a clear light blue solution obtained. The digestion mixture was then transferred to 100 ml capacity volumetric flask, cooled to room temperature and the volume was made to 100ml by adding the distilled water. 5-10 ml of this solution was taken in a distillation flask, to which 15-20ml of NaOH solution (40% w/v) was added. Thereafter, about 25 ml of the distillate (ammonia librated) was collected in 5ml, 2% boric acid that was previously mixed with two drops of mixed indicator (1% methyl red and 1% bromocresol green in 1:4 ratios). The boric acid was titrated against standard N/70 H2 SO4 to a pink end point.

$$\text{Crude protein (\%)} = \frac{V \times 0.00014 \times D \times 100 \times 6.25}{W \times A}$$

Where,

V = Volume of 2% boric acid taken – volume of N/70

H2SO4 used (ml of titrant used)

D = Dilution factor (volume made in volumetric flask)

W = weight (g) of the sample

A = aliquot taken

4.3.2 LIPIDS

Fish lipid content varies according to species, age, season and location and it can range from around 0.5% to 20% w/w or more in the wild.BCF values on a wet weight basis (BCFW) increase with increasing lipid contents. Normalization of BCF values to lipid content is one way to reduce variability when comparing measured BCF values for instance for different species or animals of different life stages. Lipid contents are commonly used to calculate BCF values on a per cent lipid basis (BCFL) and can be further used to calculate a normalized whole body BCF assuming a fixed whole body lipid content. A default value of 5% is most commonly used as this represents the average lipid content of the small fish used in OECD 305 including rainbow trout (Oncorhynchus mykiss) Bluegill sunfish (Lepomis macrochirus), zebrafish (Danio rerio) fathead minnow (Pimephales promelas) and common carp (Cyprinus carpio).

Fish lipid contents should be always measured and reported together with the calculated BCF values. The interviews revealed that fish lipid contents are usually measured and reported but that BCFs are not necessarily further normalized on a lipid basis. Only one lab mentioned that BCF values are normalized to a default value of 6% which represents the average lipid content of bluegill sunfish (Lepomis macrochirus) used in their bioaccumulation studies. A common default value (e.g. 5%, as described above) should be defined in the revised OECD guideline to give a clear basis for the comparison of BCF values across studies and among species.

Crude Fat

Crude fat was determined by using soxhlet apparatus. 1.0 g of dried sample was taken in a pouch made of Whatman filter paper (No.40) and the same was placed in a thimble connected with

soxhlet apparatus. The initial weight of the soxhlet flask was recorded and gradually filled up with the 200 ml of petroleum ether (boiling point 60-80 ^{0}C). The total apparatus was then placed over a mantle and the petroleum ether was allowed to boil for 6-8 hours for circulation through thimble by the siphon process. After boiling, the flask was taken out and the petroleum ether was allowed to evaporate. The crude lipid was determined by the difference between the final and initial weight.

4.3.3 HAEMATOLOGICAL ANALYSIS OF FISH BLOOD

Fish blood is sensitive to pollution-induced stress, and changes to the haematological parameters, such as hemoglobin content, haematocrit and the number of erythrocytes, can be used to monitor stress caused by pollutants such as heavy metals.

Hematological parameters are used as an index to detect physiological changes in a number of fish species and to assess structural and functional health during stress conditions.

Hematological analyses are commonly used to evaluate fish health and welfare in aquaculture, veterinary practice and scientific research. Hematological parameters were proved to be highly sensitive to various environmental factors including nutrition, water quality, stress or pathogens.

Analysis of the peripheral blood of fish serves for diagnostic purposes; apart from this main purpose, it is also used at present to examine the effect of toxic substances on the fish, to evaluate the condition of the fish, to evaluate the non-specific resistance of different fish breeds and strains and of the brood fish, to assess the suitability of feeds and feed mixture pellets, to evaluate the effect of stress situations etc. The recommended methods described here include only well-tested and well-proven procedures and techniques of the determination of the different haematological parameters. The values of the different haematological parameters are significantly influenced by endogenous and exogenous factors, so it is not easy to determine their physiological range. Hence, the haematological and biochemical data given in the different chapters should only serve for rough orientation.

Blood sampling

Blood is sampled for ichthyohaematological examination as soon as the fish are taken out of the environment in which they have lived. The main criteria for selection a particular method of sampling include the size of the fish, the amount of blood needed and the further fate of the fish caught for different examinations.

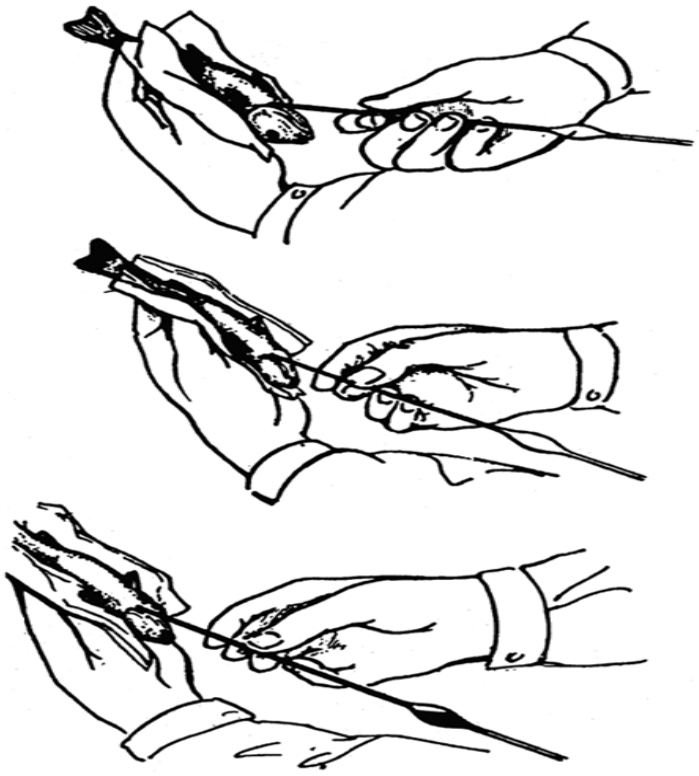
Blood sampling in fish fry

Blood from fry at an individual weight of at least 8 grammes can be sampled by the methods of cardiopunction, using a glass capillary long about 200 mm whose inner surface is lined with a fine film of heparin before use. Lift the fish, fixed head down, to the level of the eyes and apply the tip of the capillary at an angle of about 60° (in relation to the longitudinal axis of the fish body) about 1-2 mm cranially from the midpoint, which is the point of intersection of the longitudinal axis of the body with the line connecting the cranial edges of the base of both pectoral fins. At this point the carp fry have the so-called stigma: a shallow, usually pigmented depression in the skin up to 1 mm wide. Now drive the tip of the heparinized blood-collecting capillary quickly through the body wall to the pericardium and further to the heart (Fig. 21). Blood, appearing in the capillary, is the most reliable evidence that one of the cavities of the heart has been hit. When the collection is finished, pull the tip of the capillary out of the wound, hold the capillary in horizontal position and then turn it upside down several times to let the blood mix well with the anticoagulant mixture.

In heavier fish fry, for example at a weight of about 20 g, the blood collecting capillary may be replaced by a dry and clean heparinized injection needle. Such needles are driven into the heart in the same way as the glass capillary. The cardiopunction technique can also be used with older fish: this is so in those cases when the fish can be killed.

Collecting blood from fish, weighing above 200 g, including brood fish

In these fish, the best method is that of collecting blood by punction of the caudal blood vessels (Fig. 22). On the caudal peduncle ventral side the unpaired dermal scale is removed in caudal direction from the anal fin base. Within the central plane, about 1 cm caudally from the anal fin, a sufficiently long needle is introduced, firmly held on the cone of a heparinized disposable syringe, into the fish body in a craniodorsal direction at an angle of 45°. The described method is fully recommendable, mainly in larger series of blood collection; collection of 2 ml of blood from cyprinids weighing above 1000 g involves no risk of loss of the treated specimens.



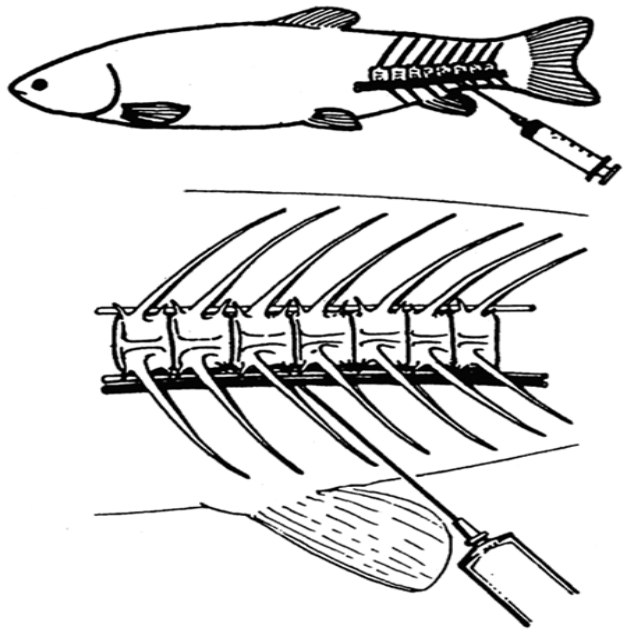


Fig. 4.1Blood sampling in fish of more than 200 g weight by the method of punction of caudal blood vessels.

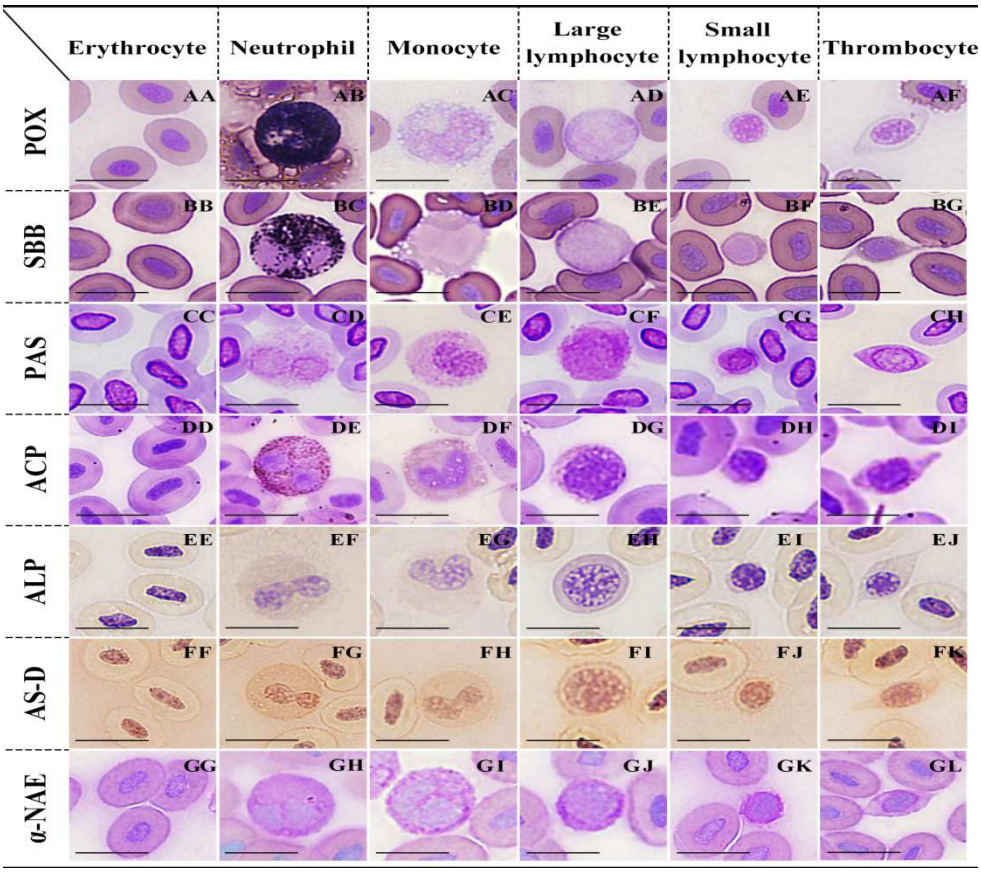
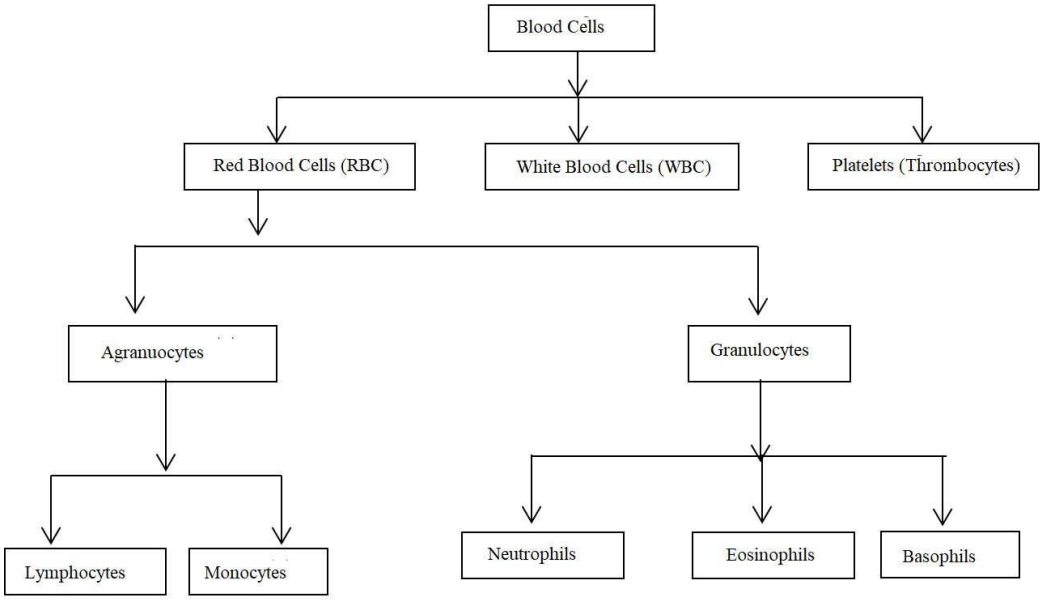


Fig.4.2 Blood cells

Stabilization of blood

Aqueous solution of heparin sodium salt is the only product used for stabilization of the fish blood. One ml of this aqueous solution contains 5000 I.U. of heparin sodium salt. 0.01 ml (about one drop) of the aqueous solution of heparin suffices to stabilize 1 ml of fish blood: the substance is left to dry on the inner surface of the test tube or flask (bublet) and the blood is collected in the test tube or bublet afterwards. A slight overdosage of heparin does not produce changes in the blood cells of the fish. Blood is a body fluid in the circulatory system of humans and other vertebrates that delivers necessary substances such as nutrients and oxygen to the cells, and transports metabolic waste products away from those same cells. Blood in the circulatory system is also known as peripheral blood, and the blood cells it carries, peripheral blood cells.



Blood is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called

RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes), and in mammals platelets (also called thrombocytes). The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas thereby increasing its solubility in blood.In contrast, carbon dioxide is mostly transported extracellular as bicarbonate ion transported in plasma.

Vertebrate blood is bright red when its hemoglobin is oxygenated and dark red when it is deoxygenated.

Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some mollusks use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen.

Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have hemocytes as part of their immune system.

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with hemo-, hemato-, haemo- or haemato- from the Greek word αἷμα (haima) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

Blood performs many important functions within the body, including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells).
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids).
- Removal of waste such as carbon dioxide, urea, and lactic acid.

- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies.
- Coagulation, the response to a broken blood vessel, the conversion of blood from a liquid to a semisolid gel to stop bleeding.
- Messenger functions, including the transport of hormones and the signaling of tissue damage.
- Regulation of core body temperature
- Hydraulic functions

4.3.4 HEMOGLOBIN IN FISH BLOOD

Haemoglobin (Hb)

The cyanohaemiglobin method is used for determination of haemoglobin in the blood of fish. The principle of the method is that haemoglobin is released from the erythrocytes and transferred to cyanohaemiglobin by means of a transformation solution; the cyanohaemiglobin is then determined photometrically. Solution after van kampen and Zijlster can be used as the transformation solution. Its composition is as follows:

potassium ferricyanide K_3 [Fe(CN) $_6$]	0.20
gpotassium cyanide KCN	0.05
gpotassium dihydrophosphate KH_2PO_4	0.14
gdistilled waterad	1000 ml

For the analysis of the blood to determine the haemoglobin, about 7 ml (or 5 ml) of the transformation solution is measured and poured into a test tube, a rinsing pipette is used to add 25 µl (or 20 µl) of fresh collected heparinized blood and the contents of the test tube are stirred immediately. The examination of heparinized blood for haemoglobin content should be performed within 24 hours after blood collection at the latest, if the blood is stored at a temperature up to 4 °C. The conversion of haemoglobin into cyanohaemiglobin is rapid: the data

can be read from the photocolorimeter after 3 minutes. The cyanohaemiglobin colouring remains stable for 24 hours at the minimum. The sample extinction measurement itself is performed in a 1 cm cell at a wavelength of 540–546 nm against the transformation solution. The haemoglobin content is determined from the calibration curve. The calibration curve is drawn in the usual way, using the cyanohaemiglobin standard and the transformation solution.

Haemoglobin is the respiratory pigment in most of the fishes and its amount varies with the total number of erythrocytes in the fish.

However, there is no haemoglobin in the Antarctic Ice fishes, which survive without haemoglobin in blood, because:

i. Their metabolic oxygen requirement is low, and the cold Antarctic water has a high level of dissolved oxygen.
ii. These fish are very sluggish.
iii. They have special adaptations,such as the large heart and blood volume which help in efficient moment of their blood.

Thus, these fishs are able and can get along without haemoglobin. Fish haemoglobin is basically of two types: monomeric and tetrameric. Hemoglobin is composed by polypeptide chains, known as globins, each having a prosthetic group called heme, identical in every fish species. On the other hand, globins differ from species to species and among isoforms. Remarkably, globins seem to occur in all organisms and tissues, exhibiting a diversity of quaternary structures and a large number of functions apart from oxygen transportation and storage, as illustrated by cytoglobins and neuroglobins In teleosts, including the Atlantic salmon, carp and zebrafish, the adult alpha globin gene is adjacently linked to the beta globin gene and the embryonaryglobins are completely different from adult globins. In vertebrates, the hemoglobin molecule includes four globin chains that create a stable tetramer formed by two alpha-like and two beta-like chains with 141 and 146 amino acid residues, respectively. Each one contains a heme group, which allows the binding of four oxygen (O2) molecules in a reversible form, according to the scheme below:

$$Hb + 4O2 = Hb(O2)4$$

deoxy-Hb oxy-Hb

The main function of hemoglobin is to transport oxygen from the gas-exchange organs to peripheral tissues. It must be able to bind oxygen strongly but at the same time to release it when necessary, depending on the partial pressure of the gas . Reversible oxygen binding is possible thanks to the heme group, specifically with the participation of an iron atom in the ferrous form, Fe2+. For some species of primitive fish such as lampreys and hagfish, reversible hemoglobin dissociation occurs in response to oxygen binding. In the case of lampreys, the oxygenated form is monomeric, undergoing associations with dimers and tetramers when deoxygenating. In Myxineglutinosa (hagfish) there are three monomeric hemoglobins in the oxygenated form, but when they release O2, there is an association to form heterodimers and heterotetramers.

Hemoglobins are particularly important in fish adaptation as they constitute an interface between the organism and the environment. Fish particularly face a very variable environment and temporal and spatial alterations in oxygen availability, in contrast to terrestrial animals. However, a variety of environmental and physiological adjustments are observed in fish exposed to environmental hypoxia in order to improve O2 transfer. Many Amazonian fish obtain O2 directly from the air when submitted to hypoxia, being obligate or facultative air breathers. The anatomical modifications that allow accessory air-breathing include changes in the gills, mouth, stomach, intestine, and vascularization of the swim bladder. Species that do not have this capacity are obliged to accomplish metabolic and behavioral changes to deal with limited O2 availability. Such adjustments involve ventilation frequency and volume, heart rate, increase in the number of erythrocytes, hematocrit and hemoglobin concentrations, changes in organic phosphate concentrations, presence of iso-hemoglobins with different functional properties, and metabolic depression .

The monomeric haemoglobin is characteristic of the Agantha (lampreys and hag fishes), and consists of a single polypeptide molecule with a molecular weight of about 17,000 daltons. Tetrameric haemoglobin is found in most higher fishes. It is composed of four chains of amino acids (two alpha and two beta chains), with the molecular weight of about 65,000 daltons. The tetrameric haemoglobins are also of many kinds, and more than one may be found in one fish.

For example, rainbow trout has four kinds of haemoglobins in its blood, the gold fish has three kinds, and the Americn eel, Anguilarostrata, has only two kinds of haemoglobin in its blood. Each type of haemoglobin has a different functional property, so that various combinations have been evolved in response to different environmental conditions.

In migratory fishes, as in the catadromus eel, one kind of haemoglobin has a high oxygen affinity in salt water, while the other type has a high oxygen affinity in fresh water. The presence of polymorphic haemoglobin is therefore, considered an adaptation to acclimatise the eels in the environments of different salinity, and the fish is able to maintain an approximately constant amount of blood oxygen.

It has been reported that goldfish, acclimatised to 2 C had two different haemoglobins, while those at 20-35C had three types. It has also been shown that the third type can be made to appear and disappear with temperature changes within a few hours. Possibly, the third haemoglobin is not synthesized fresh, and is the result of rearrangement of alpha and beta subunits in other haemoglobins.

Several factors such as the pH, temperature, CO_2concentration, and organic phosphate concentration, influence the blood oxygen affinity. Of these, pH and CO2 concentration in blood are the most important factors. A decrease in affinity for oxygen, with decreasing pH or increasing Pco2 (Bohr effect) normally serves to "drive off" oxygen from the haemoglobin, thereby raising plasma Po2 and facilitating its diffusion to surrounding tissues.

4.3.5 WBC/ RBC/DLC IN FISH BLOOD

Determination of the parameters of the red blood picture

Erythrocyte count (Er, RBC)

The erythrocyte count in fish blood is determined in heparinized blood diluted by the Hayem solution at a ratio of 1:200. The solution has the following composition:

mercury dichloride HgCl2 - sublimate	2.5 g
sodium sulphate Na2SO4	25 g

sodium chloride NaCl	5 g
distilled water	ad 1000 ml

The flask (bublet) method after Bürker is used for the dilution of the blood. The blood is diluted in special glass bublets or in penicillin phials (volume 15 to 25 ml). First, a special pipette is used to put an accurate amount of 4975 μl of Hayem solution (filtered before use) into the bublet or phial; then 25 μl of heparinized blood is added, using a flushing micropipette. The micropipette is rinsed several times by repeatedly sucking the solution, the bublet is closed with a rubber stopper and its contents are stirred by circling motion for 2 to 3 min. A dropper or a Pasteur pipette is used to fill Bürker's counting cell with the diluted blood. The red blood cells are counted in 20 rectangles, regularly distributed over the whole lattice of the counting cell. The counting is usually done at a 200-fold magnification.

The resultant counted amount of erythrocytes is then reduced 100 times and the resultant value is the number of erythrocytes in $T.1^{-1}$ (Tera = 10^{12}). The erythrocyte count can still be determined by the traditional method after 24 hours of storage of heparinized blood at a temperature of up to 4 °C.

Colorimetric methods of determining the erythrocyte count in the blood of fish are introduced at present. The colorimetric method of determining the erythrocyte count after Pawinski, compared with the traditional method, is simpler, more rapid, and less laborious, does not bear a subjective error, has a greater reproducibility, and is suitable for series determinations. The disadvantage is that its use in ichthyotoxicology is limited, especially in the study of the action of substances that increased the mean corpuscular volume.

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