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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.Tech. BIOTECHNOLOGY

UNIT – I - MARINE BIOTECHNOLOGY – SBT1304

UNIT I: Introduction to marine microbes in the ocean (12 hrs)

Physical and chemical properties of sea water, Zonation of sea: Euphotic – mesopelagic – bathopelagic- benthos - deep sea. Marine ecosystems and biodiversity: Diversity & adaptation. Marine microbial diversity: Marine microbial habitats - Microbial distribution in the oceans - Factors that impact marine microbial diversity - Interactions between marine microbes and invertebrates - Marine viruses

PHYSICAL AND CHEMICAL PROPERTIES OF SEAWATER

The physical and chemical properties of seawater vary according to latitude, depth, nearness to land, and input of fresh water. Approximately 3.5 percent of seawater is composed of dissolved compounds, while the other 96.5 percent is pure water. The chemical composition of seawater reflects such processes as erosion of rock and sediments, volcanic activity, gas exchange with the atmosphere, the metabolic and breakdown products of organisms, and rain. (For a list of the principal constituents of seawater, *SEE* seawater: Dissolved inorganic substances.) In addition to carbon, the nutrients essential for living organisms include nitrogen and phosphorus, which are minor constituents of seawater and thus are often limiting factors in organic cycles of the ocean. Concentrations of phosphorus and nitrogen are generally low in the photic zone because they are rapidly taken up by marine organisms. The highest concentrations of these nutrients generally are found below 500 metres, a result of the decay of organisms. Other important elements include silicon (used in the skeletons of radiolarians and diatoms; *SEE* Figure 2) and calcium (essential in the skeletons of many organisms such as fish and corals).

The chemical composition of the atmosphere also affects that of the ocean. For example, carbon dioxide is absorbed by the ocean and oxygen is released to the atmosphere through the activities of marine plants. The dumping of pollutants into the sea also can affect the chemical makeup of the ocean, contrary to earlier assumptions that, for example, toxins could be safely disposed of there.

The physical and chemical properties of seawater have a great effect on organisms, varying especially with the size of the creature. As an example, seawater is viscous to very small animals (less than 1 millimetre [0.039 inch] long) such as ciliates but not to large marine creatures such as tuna.

PHYSICAL AND CHEMICAL PROPERTIES OF THE OCEAN

Terrestrial habitats exhibit extreme ranges in temperature and receive varying amounts of sunlight, precipitation, and wind. Additionally, they have other unique chemical and physical properties that make them suitable places for one species to live, but completely uninhabitable for another. So, too, oceanic habitats exhibit chemical and physical properties that make certain ocean zones suitable or unsuitable places for different species to live. In fact, chemical and physical properties of the ocean are crucial to the survival of marine organisms. This chapter addresses the chemical (salinity and dissolved gases) and physical (temperature, density, buoyancy, waves, tides, and currents) properties of ocean water that are delicately intermingled to produce one of the most self-sustaining life support systems on earth.

A. Salinity

The ocean is salty. But what makes it salty when the water flowing into it is from freshwater rivers, streams, and precipitation? Freshwater rivers and streams weather, or slowly wear away, the rocks and soils they flow over as they make their descent from mountainous and other inland regions toward the ocean. Rocks and soils release inorganic salts and other chemical compounds as they are weathered by this continuous flow of water. These inorganic salts and other chemical compounds are finally deposited in the oceans at the end of their journey from far away inland places. Additionally, precipitation causes fresh water and chemical compounds to be released from the atmosphere into the oceans.

Some of the inorganic salts and other chemical compounds become dissolved in the ocean water once they reach the ocean. Sodium(Na+), chlorine (Cl-), magnesium (Mg2+), and calcium (Ca2+) are inorganic salts that make up most of the solid material that has become dissolved in the oceans (Table 2-1). Ocean water is approximately 96.5% pure water and 3.5% naturally-occurring dissolved substances.

Constituents of seawater.

Constituent	Symbol	% by Weight
chloride	Cl^-	55.1
sodium	Na^+	30.6
sulfate	$SO4^{2-}$	7.7
magnesium	$_{Mg}2+$	3.7
calcium	Ca^{2+}	1.2
potassium	$_K+$	1.1
Total		99.4

Salinity is the term used to define the total amount of dissolved inorganic salts in the ocean. Salinity is measured, in most cases, in parts per thousand (ppt or ‰). For example, a salinity of 1‰, or 1 ppt, is equivalent to 1 gram of salt in 1,000 grams of pure water; a salinity of 30‰, or 30 ppt, is equivalent to 30 grams of salt in 1,000 grams of pure water. There are a variety of different factors influencing the relative amounts of dissolved inorganic salts in the ocean. Sunlight, for example, causes only the fresh water part of the ocean to be evaporated, or absorbed by, the atmosphere, leaving only the inorganic salts behind. Frequent precipitation, on the other hand, adds fresh water back into the ocean system, thereby diluting the relative concentrations of inorganic salts in ocean water. Salinity can be varied by (1) changing

The concentration of salts in the ocean, and/or (2) changing the concentration of water in the ocean. Rates of evaporation and precipitation can thus be related to salinity, with areas of generally high evaporation having high salinities and areas of high precipitation generally having lower salinities. Although the amount of dissolved inorganic salts varies among different areas of the world's ocean, the relative proportions of the inorganic salts themselves remain very similar throughout.

Salinities in the ocean range from less than 5‰ where rivers begin to reach coastal areas to as much as 45‰ in the saltiest oceans. The Black Sea has a relatively low salinity of 18‰, while salinities in the Red Sea, one of the saltiest seas in the world, range from 40 to 42‰. The salinity of open waters of the Atlantic Ocean averages 35‰, but may be as low as 15 to 25‰ in harbors, sounds, and bays, to about 30‰ along the coast. Salinity increases as the distance from shore increases, with salinities in continental shelf waters off the Southeastern U.S. ranging from 30 to 36‰, from 36 to 36.2‰ in the Gulf Stream, and from 36.2 to 37‰ in waters transported by currents from the Sargasso Sea. The global distribution of sea surface salinities varies substantially (Fig. 2-1). This variability is mostly due to the relative amounts of precipitation and evaporation or the addition or removal of atmospheric fresh water. In tropical equatorial regions, evaporation is approximately equal to precipitation, and we observe a salinity of 34.5‰. Between latitudes 20 and 40° in both hemispheres, evaporation exceeds precipitation, resulting in high surface water salinities, reaching 35.7‰ (and higher). Near the polar regions (latitudes higher than 60° N and S), precipita tion is significant and dilutes the seawater, resulting in much lower salinities (<33‰). The salinity of different ocean areas is a major factor in determining the types of organisms capable of living there. As you will see in the following sections, interactions between salinity and temperature affect other physical properties of ocean water. Salinity also serves as one of the driving forces of major oceanic current systems

B. Temperature

Temperature is one of the most important physical factors affecting the distribution of life in the oceans. Additionally, temperature controls the rate at which organisms metabolize, or break down, food items into nutrients that they can use. Exchange of gases, such as oxygen (O2) and carbon dioxide (CO2), in the marine environment is greatly affected by temperature. Ocean temperatures also

affect the survival of organisms as they develop through various life cycle stages, such as egg, larval, and juvenile stages. Sea surface temperature in the ocean ranges from very warm in the tropics to below freezing in the polar regions. Oceanic waters become warmer as one moves toward the equator and conversely, cooler as one moves toward the poles. Ocean surface temperatures generally range from 0 to 30°C (32 to 86°F). Because salt lowers the freezing point of pure water, which is 0°C (32°F), ocean water freezes at about -1.1°C (30°F). Just as inorganic salts are left behind in the ocean water when freshwater is evaporated into the atmosphere, only the freshwater portion of the ocean surface freezes, thereby leaving the ocean water beneath the frozen surface layer saltier. The temperature of the Atlantic Ocean ranges from -2°C to greater than 30°C (28.4 to 86°F) (Fig. 2-2).

Surface temperatures in the ocean also vary seasonally, with the greatest differences in seasonal temperatures occurring near the poles. Temperatures remain relatively unchanged near the equator. Off the Southeastern U.S., ocean temperatures over the continental shelf can range from 9 to 25°C (48 to 77°F) at the surface and from 9 to 23°C (48 to 73.4°F) at the bottom during winter months to 27 to 30°C (80 to 86°F) at the surface and 20 to 28°C (68 to 82°F) at the bottom during summer months. Generally, the deep ocean is very cold, and fewer organisms are capable of surviving these cold temperature extremes. But the recent discovery of hydrothermal vents along the ocean floor has revealed that heat is released from the earth's interior through fissures located on the ocean floor. These fissures and hydrothermal vents are most often located at the edges of divergent lithospheric plates (described in Chapter 1) and provide an oasis of warm water in the cold, deep ocean.

Most ocean waters have a subsurface temperature feature known as a thermocline. A thermocline is an area in the water column of the ocean where temperature changes very rapidly (Fig. 2-3). Thermoclines sepa separate warmer surface waters from the cooler waters below. Because thermoclines are physical features that separate warmer waters from colder waters, they can be very effective barriers across which gases, nutrients, and in some cases, organisms, move. The vertical location of the thermocline can change seasonally.

Variations in density, or the ratio of mass to volume, of the ocean are a function of salinity and temperature. Oceanic waters with higher salinities are more dense than oceanic waters with lower salinities. In other words, a liter of water with a salinity of 36‰ weighs more than a liter of water with a salinity of 32‰. Additionally, waters that have cooler temperatures have higher densities than waters with warmer temperatures. Ocean waters with higher salinities and cooler temperatures have the greatest densities. Dense water masses actually "sink" toward the ocean floor, while less dense ocean water masses "float" at or near the ocean's surface.

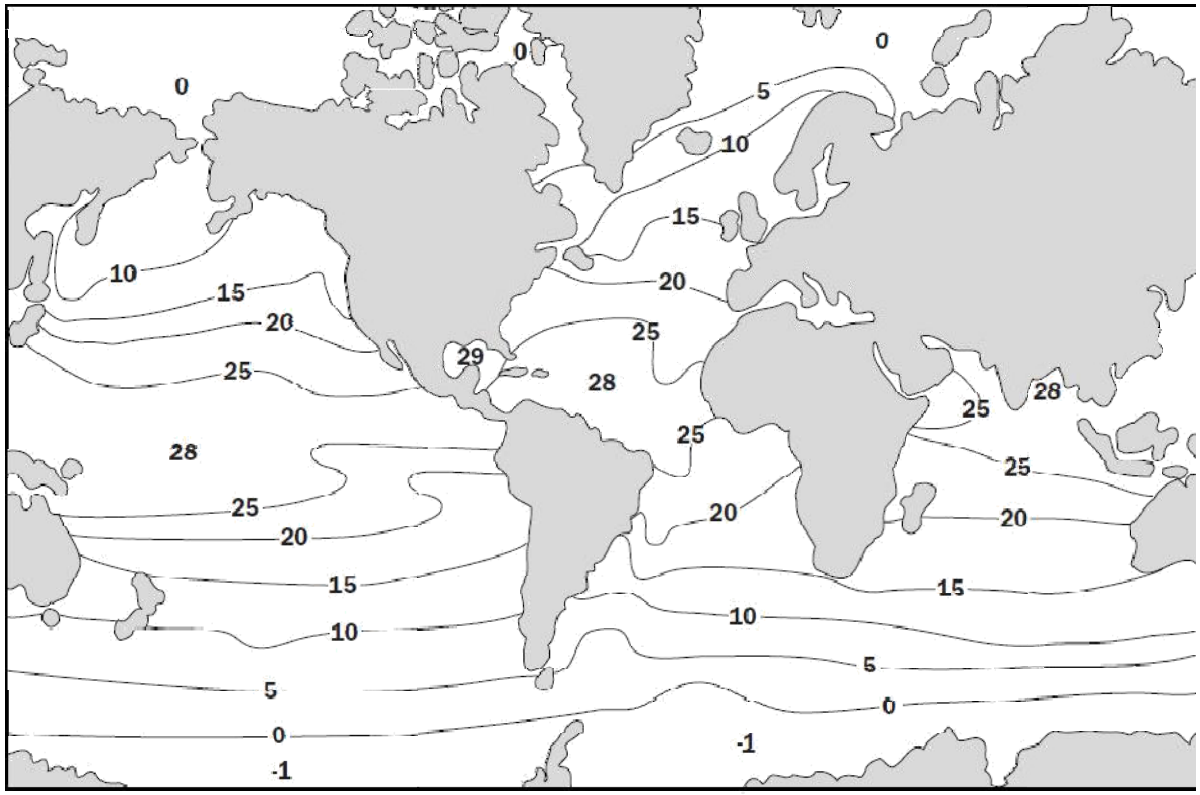


Figure 2-2. *Worldwide surface temperature distribution (in °C).*

Density

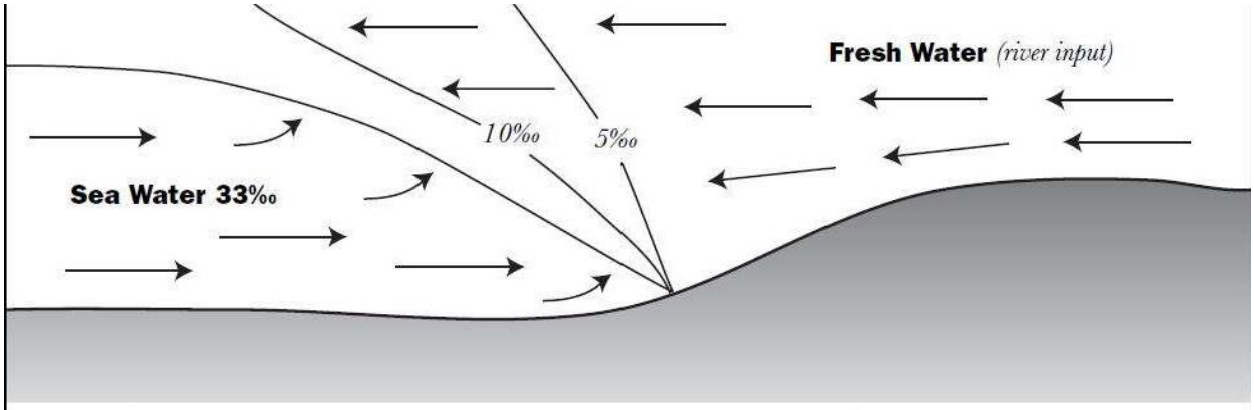


Figure 2-4. *Fresh water entering an estuary from a river is less dense than the salt water. In some estuaries, a wedge of denser salt water is created as the fresh water overrides denser water.*

In coastal areas, fresh water in a river tends to flow toward the ocean along the river's surface, while the more dense salt water flows upstream along the bottom of the river (Fig. 2-4). The degree of mixing between the two water masses varies, depending on river flow, tides, wind, and the width and depth of the river as it approaches the ocean.

At the beginning of this chapter, we discussed that unique chemical and physical properties, like salinity and temperature, vary somewhat among the different ocean basins. Water masses from each ocean basin must ultimately meet since all of the major ocean basins are interconnected and form one global ocean.

The ocean is, therefore, made up of "layers" of different water masses that are continually sinking toward the ocean floor or rising toward the ocean surface, depending on their indi vidual densities. It is the interactions among factors occurring at the ocean's surface, such as freezing, evaporation, precipitation, heating, and cooling, that determine the density of a certain water layer and thus, its vertical position in the "layered" global ocean.

C. Buoyancy

Just as water masses with different densities either sink below or float on top of one another, objects that are denser than water sink while objects that are less dense than water float. Buoyancy is defined as the ability to remain afloat in a liquid. Because salt water is more dense than fresh water, salt water provides greater buoyancy to an object floating on the surface than does fresh water. A person or a boat is more buoyant in salt water than in fresh water (Fig. 2-5). Denser liquids have a greater buoyancy force, or the force that makes an object float. In order for an object to float in a liquid, it must be less dense than that liquid. Some organisms living in the ocean float on top of the ocean's surface. These organisms are very buoyant, or less dense, than the sea water in which they live, and most of their body mass is, in fact, made up of water. Some of these organisms have specialized structures that make them more buoyant, such as the balloon-like floats of the Portuguese man-o- war or the air sacs of *SARGASSUM*, a brown alga common in the Sargasso Sea of the Atlantic Ocean (Fig. 2-6). *SARGASSUM* occasionally can be found washed ashore along the Southeastern U.S. coast. It can also frequently be found floating offshore in the Gulf Stream and makes up the "weed line" to which offshore fishermen often refer.

Oil floats on the surface of the water and many marine organisms produce an oil that makes them more buoyant. Even fish eggs may contain oil droplets, which enable them to remain at the surface or suspended in the water column. Increased body surface area and other unique adaptations, such as elongate spines and antennae, also retard the rate of sinking.

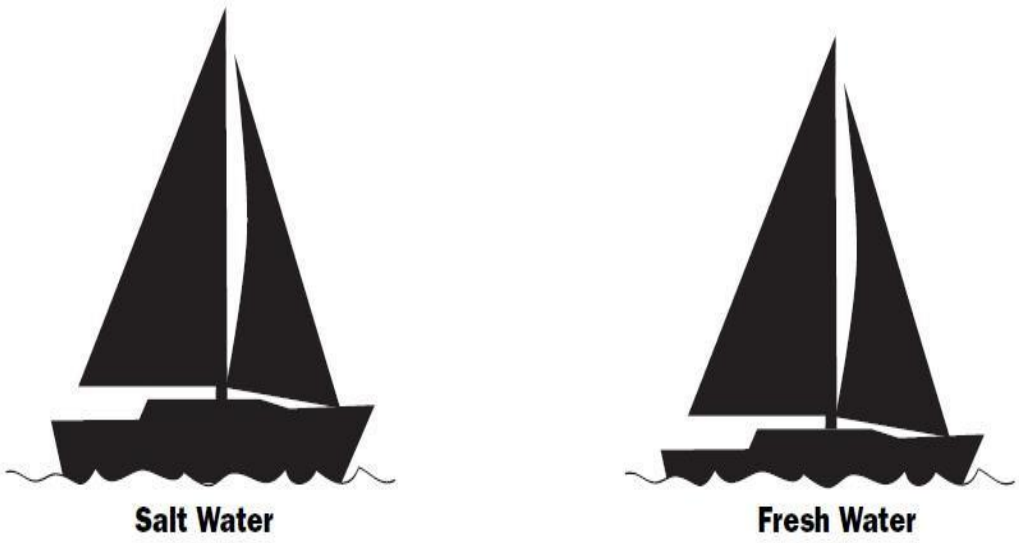


Figure 2-5. *An object is more buoyant in salt water than in fresh water.*

D. Nutrient Uptake and Gas Exchange

The ocean provides a medium for uptake of nutrients and gases and elimination of wastes for all of the organisms living in it. Plants living in the ocean need "fertilizers," such as nitrate (NO_3^-) and phosphate (PO_4^{2-}), for continued growth and survival just as terrestrial plants do. Marine plants get nitrate and phosphate from the ocean water that surrounds them. Marine plants also need carbon dioxide (CO_2) to make their own food through the process of photosynthesis Still other marine organisms need magnesium (Mg^{2+}), calcium (Ca^{2+}), silica [$Si(OH)_4$], and bicarbonate HCO_3^-) for production of their protective shells. Other marine animals need oxygen (O_2) to breathe, and they give off carbon dioxide (CO_2) just as we do here on land.

E. Waves and Tides

Tides are the rise and fall of sea level that is caused by the gravitational pull of the moon and the sun on the Earth.

Waves are actually energy that moves across the surface of the water. In the scientific community, this is more commonly known as wind waves as these waves are generated by wind.

Waves

Wind is a form of energy.

Wind energy blowing along the surface of the ocean is transferred to the ocean as waves and currents. Waves originate in the open ocean and, in many cases,the waves we see along the coast were generated far away at sea Waves can be so small that they are hardly noticeable.

One of the largest waves ever recorded was 34 meters high (112 feet)!

Earthquakes, submarine landslides, and volcanic eruptions also produce waves by displacing the water, thereby setting it in motion in the form of a wave.

The wave height increases as the water depth decreases.

An estimated 8,000 waves a day hit an average coastal beach.When ocean waves reach coastal shorelines, large amounts of energy are transferred from the wave to the beach and erosion of the land often takes place.

Tides

Tides, or the periodic rise and fall of the ocean's surface, are caused by the gravitational pull of the moon and the sun on the earth.

Because the moon is much closer to the earth than the sun, its gravitational pull on the earth is much greater than that of the sun.

The moon's gravitational attraction "pulls" the ocean covering the earth's surface toward the moon, creating a bulge of water at the point on the earth directly facing the moon (Tidal bulge). There is a second tidal bulge on the side of the earth that faces away from the moon. This bulge is the result of the moon's revolution around the earth.

Tidal ranges have been classified into three groups: microtidal, mesotidal, and macrotidal. These groupings were originally defined using the measurement unit of feet rather than meters.Tidal ranges between 0 and 6 feet as microtidal; between 6 and 12 feet as mesotidal; and greater than 12 feet as macrotidal.

Organisms living in intertidal areas, or areas that are exposed to air during low tides, have developed special adaptations that enable them to live underwater during high tide, and completely exposed during low tide.

Movement of water along the surface of the open ocean, known as surface current circulation, is primarily caused by wind.

Surface currents are slow, broad currents, the effects of which can extend to depths of 200 m (656 feet).

Ocean currents also have major effects on weather patterns throughout the world.

Surface Currents: Each surface current has its own unique temperature, salinity, density, directional flow, speed, and well-defined boundaries between adjacent currents.

Thermohaline Circulation: Thermohaline circulation is caused by the vertical movement of water as a result of temperature and salinity differences. Thermohaline circulation is the major factor driving deep ocean current patterns.

Cold dense water masses will sink below layers of ocean water.

This phenomenon of “sinking” water layers, or Downwelling, drives thermohaline circulation in the deep ocean.

It is the combination of salinity and temperature of a

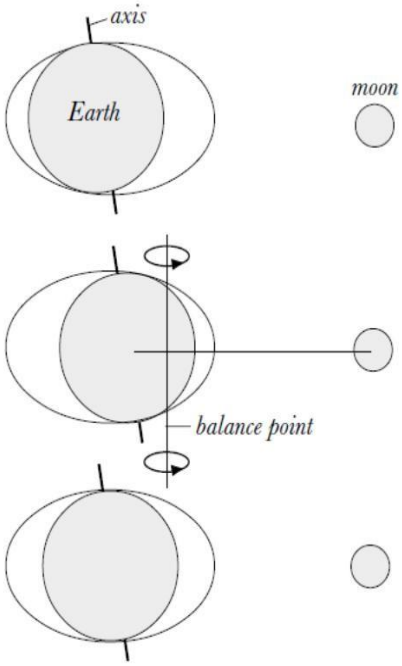
particular water mass that determines its vertical position, or the depth to which it “sinks,” in the water column

Upwelling is the mechanism by which deep ocean waters rise toward the surface.

Upwelling in the open ocean is in part driven by thermohaline circulation which displaces and “pushes” bottom waters upward as the denser water masses sink to the bottom.

The upwelled bottom waters are rich with nutrients that have accumulated from the constant rain of recycled material to the sea floor.

These nutrients enter the photic zone and microscopic plant life flourishes, providing food for a vast number of animals living in surface waters.



a) Effect of gravitational force.

The gravitational pull on the earth by the moon causes a tidal bulge on the side of the earth that faces the moon.

b) Effect of centrifugal force.

The earth-moon system rotates around a "balance point," or axis for the moon's revolution around the earth. Centrifugal force creates a tidal bulge on the side of the earth that faces away from the moon.

c) Combined effects of gravitational and centrifugal forces.

Two tidal bulges result from the combined influences of gravitational and centrifugal forces.

Figure 2-9.

Gravitational (a) and centrifugal (b) forces between the earth-moon system contribute to the formation of two tidal bulges (c), one on each side of the moon.

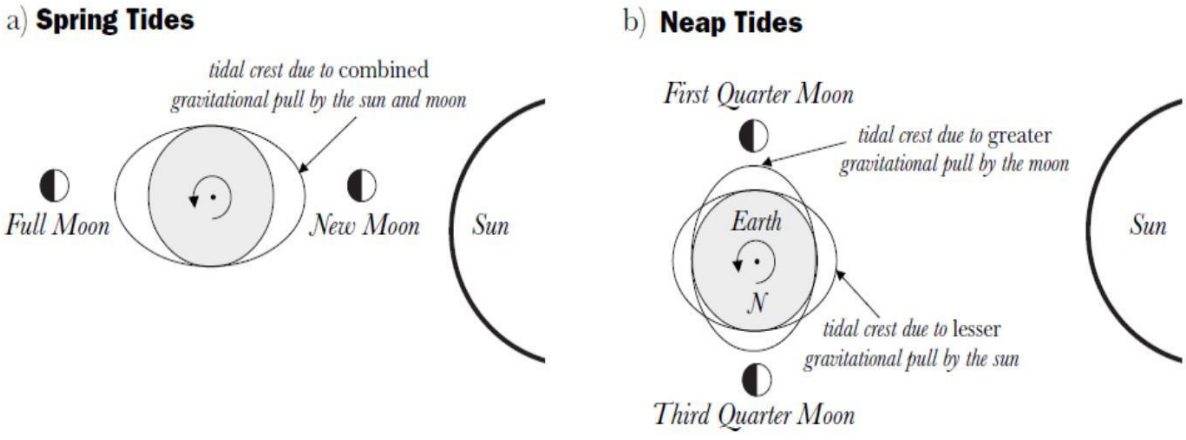


Figure 2-12.

(a) Spring tides are the result of two combined tidal crests from the moon and sun. The effect is to accentuate the difference between high and low tides. (b) Conversely, neap tides are the result of two opposing tidal crests from the moon and sun. The effect is to dampen the difference between high and low tides.

F. OCEAN CURRENT

An ocean current is any more or less permanent or continuous, directed movement of ocean water that flows in one of the Earth's oceans.The currents are generated from the forces acting upon the water like the earth's rotation, the wind, the temperature and salinity differences and the gravitation of the moon.

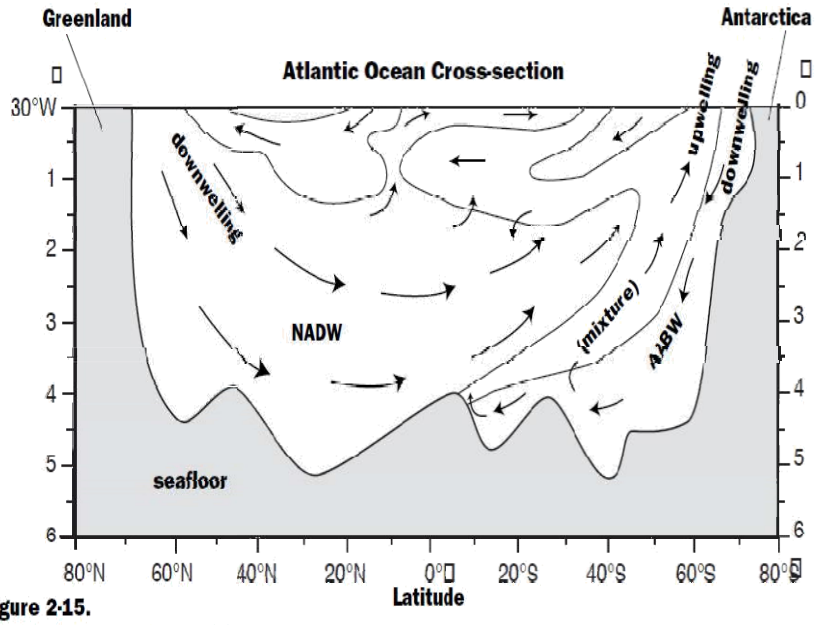


Figure 2-15.

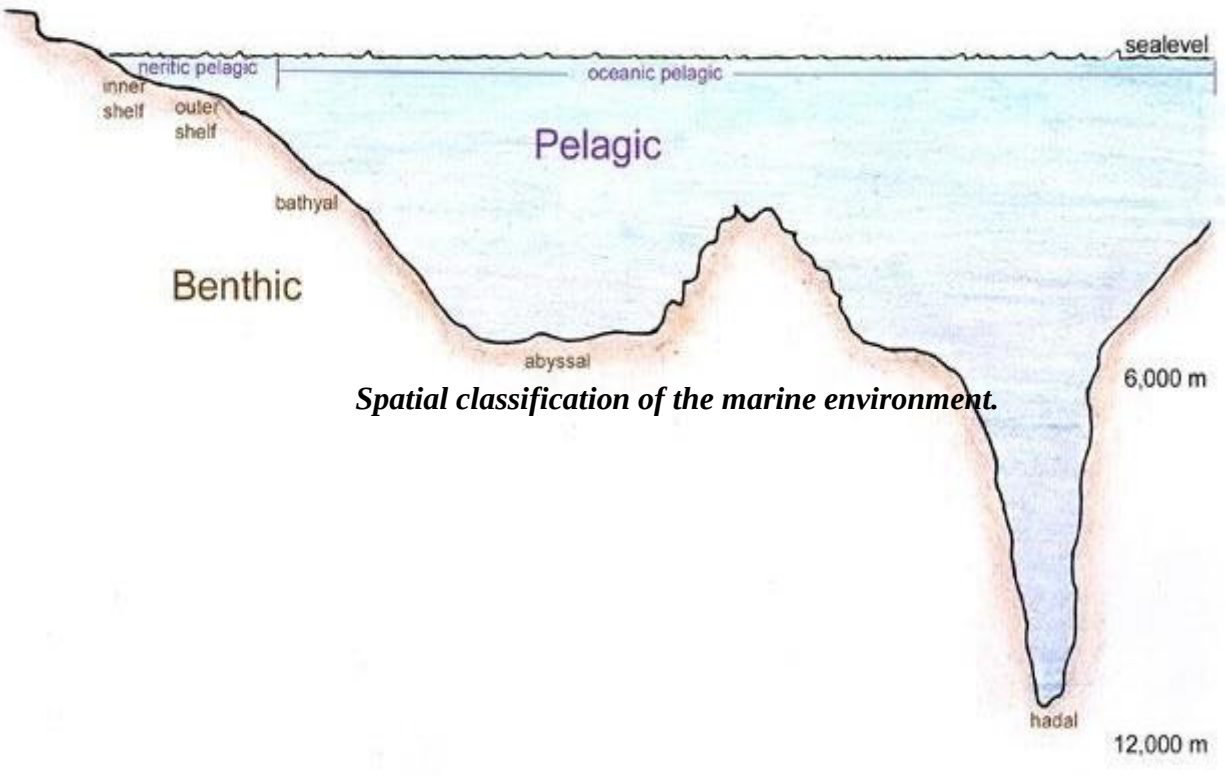
Simplified drawing of density–driven, thermohaline circulation of the North Atlantic Deep Water (NADW) and the denser Antarctic Bottom Water (AABW).

The depth contours, the shoreline and other currents influence the current's direction and strength. Ocean currents can flow for thousands of kilometers. They are very important in determining the climates of the continents, especially those regions bordering on the ocean.

Perhaps the most striking example is the Gulf Stream, which makes northwest Europe much more temperate than any other region at the same latitude. Deep ocean currents are driven by density and temperature gradients. Thermohaline circulation, also known as the ocean's conveyor belt, refers to the deep ocean density-driven ocean basin currents. These currents, which flow under the surface of the ocean and are thus hidden from immediate detection, are called submarine rivers.

THE WORLD OCEANS: CLASSIFICATION OF MARINE ENVIRONMENTS

Because of the variations in the three dimensional ocean and the changes in light, there are two primary ways of classifying marine environments. One way is by space and the other way is by light.



Spatial classification of the marine environment.

Being pelagic means to be in the water, surrounded by water at any depth. The pelagic division above the continental shelf is distinguished as the neritic province from the pelagic area above the open ocean (abyssal plain, oceanic ridges/rises, and trenches) which is call the oceanic province. Pelagic organisms that can swim relatively well are called nektonic pelagic (or just nektonic) whereas those that cannot swim, or are feeble swimmers, are called planktonic pelagic (or just planktonic).

Being benthic means to be on the bottom or on a solid surface. Most benthic habitats are associated with the bottom of the ocean and are distinguished by depth as follows: inner shelf (closest to the continent), outer shelf (along the outer edge of the continental shelf next to the continental slope), bathyal zone (on the continental slope *Spatial classification of the marine environment.*

averaging between 200 to 3-6,000 meters), abyssal zone (on the abyssal plain, the relatively flat deep-sea area that may be 3-6,000 meters deep), and hadal zone (on the bottom of the trenches - below 6,000 m to 11,000+ meters). Benthic is also used to describe organisms that live on other organisms, like the barnacles that live on some species of whales. The whales are pelagic but their barnacles are benthic.

Some species of marine life can be both pelagic and benthic at the same time. An example of this is the many flatfish (like halibut, sole, and flounder). They tend to rest on the bottom (and are thus benthic at that time) but can swim through the water in search of food (and are pelagic while swimming).

Some species of marine life can be both pelagic and benthic at different times of their life. This is common for most of the benthic invertebrate animals. They begin life as pelagic (water dwelling) babies but settle after a period (often weeks or months) and become benthic adults. Most seastars, sea urchins, snails, clams, crabs, lobsters, corals and sea anemones have this life style.

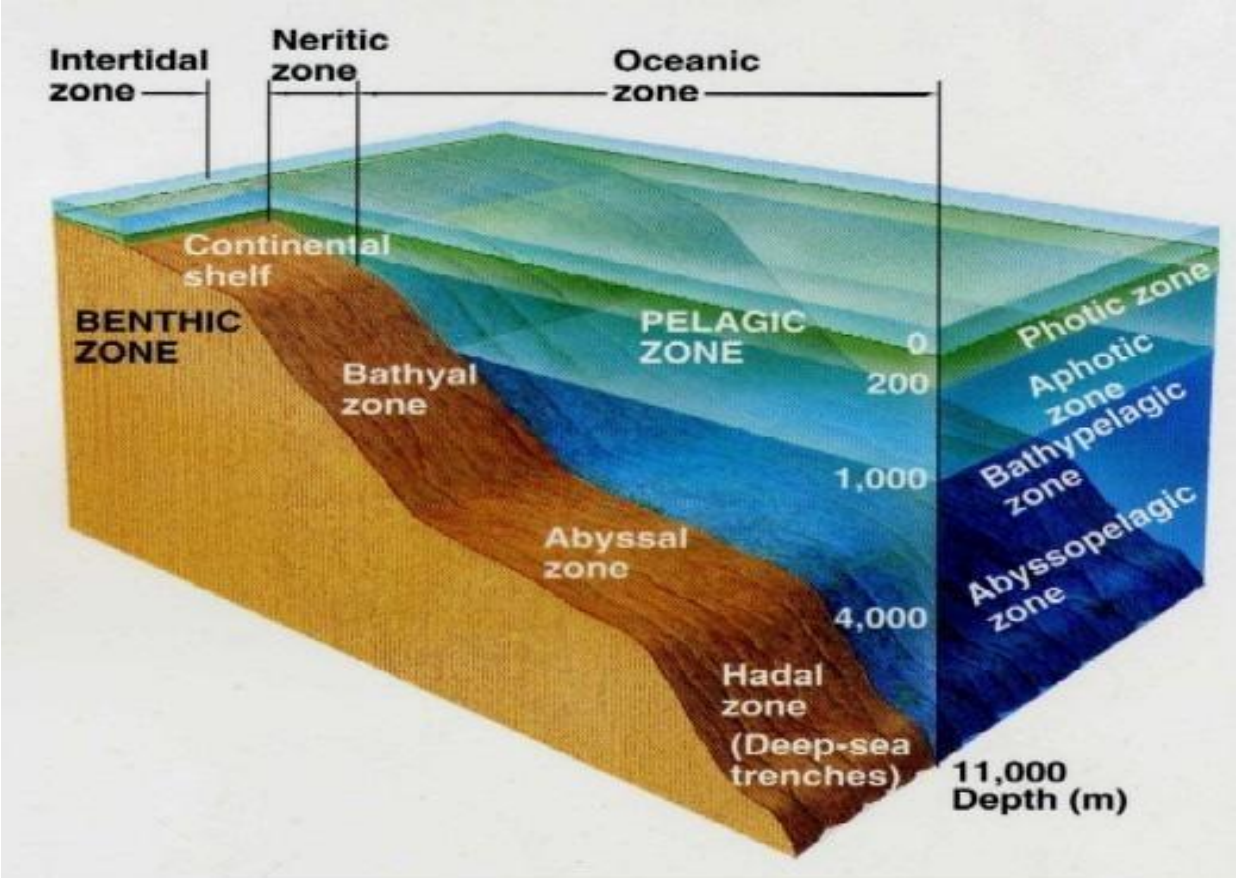
Using light the marine environment is divided into the photic zone and aphotic zone. The photic zone is the area where there is enough light for photosynthesis to occur. It changes with the seasons, latitude, time of day, clarity of the water, as well as with the weather. In general the photic zone would not be found below 200 meters and would normally be well above this. The aphotic zone is below the photic zone and is the place where there is no photosynthesis.

With all of these classifications there are many ways to describe the marine environment. The oceans are one of the most diverse environments on Earth with incredibly interesting life forms. Many people say we know more about the surface of the moon than we do about the ocean floor and this is proved by the constant new discoveries made by marine scientists.

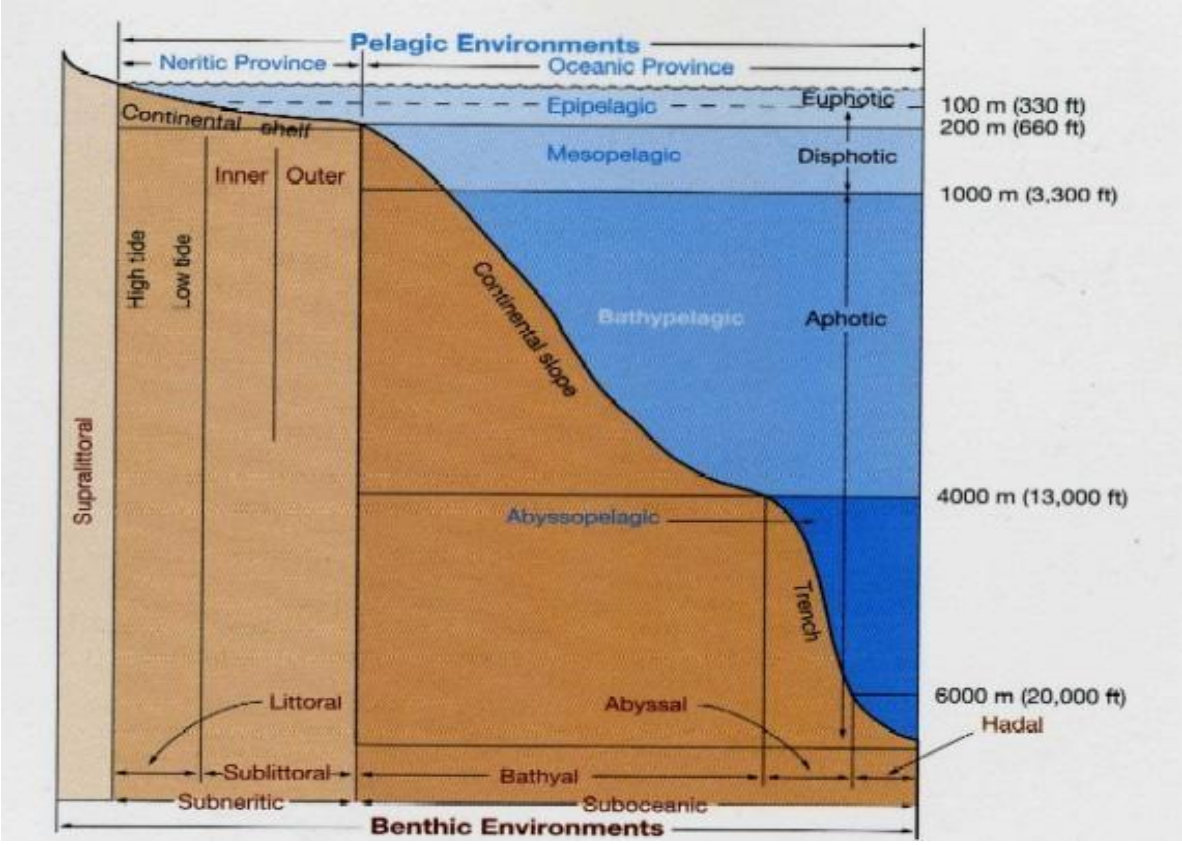
MARINE ENVIRONMENT AND PRIMARY PRODUCTIVITY

Classification of the Marine environment

Marine scientists divide the ocean environment into zones. Marine Zones are areas with uniform physical conditions. Common classifications are based on physical factors such as depth, light, temperature, salinity, etc. The most basic zonation is based on substrate: exclusively water environment (pelagic) and bottom interface (benthic).



The pelagic zone is divided by depth into: nerithic zone, which includes the nearshore areas over the continental shelves; and the oceanic zone, the areas seaward of the continental shelves. The oceanic zone is further divided into epipelagic zone (same as photic zone), mesopelagic, bathypelagic, and abyssopelagic zones. Abyssopelagic zone is water in the deep ocean trenches. The last three zones are all at aphotic depths.



The shallowest benthic environments (below the neritic zone) are:

* Supralittoral - bottom substrate above high tides (not part of ocean).

* Littoral - bottom substrate within the intertidal zone.

* Sublittoral - bottom substrate below the lowest
tides. Beyond the continental shelf break are:

* the Bathyal zone (ocean bottom down to the abyssal plain or the average depth of the ocean floor),
* the Abyssal zone (from 4,000 - 6,000 m depths), and

* the Hadal zone representing the deepest ocean bottom in the deepest trenches.

Physical factors affecting Marine Life Any factor of the physical environment that affects the survival of marine organisms are physical factors. These physical factors form barriers between various communities of marine organisms. The most important of these are:

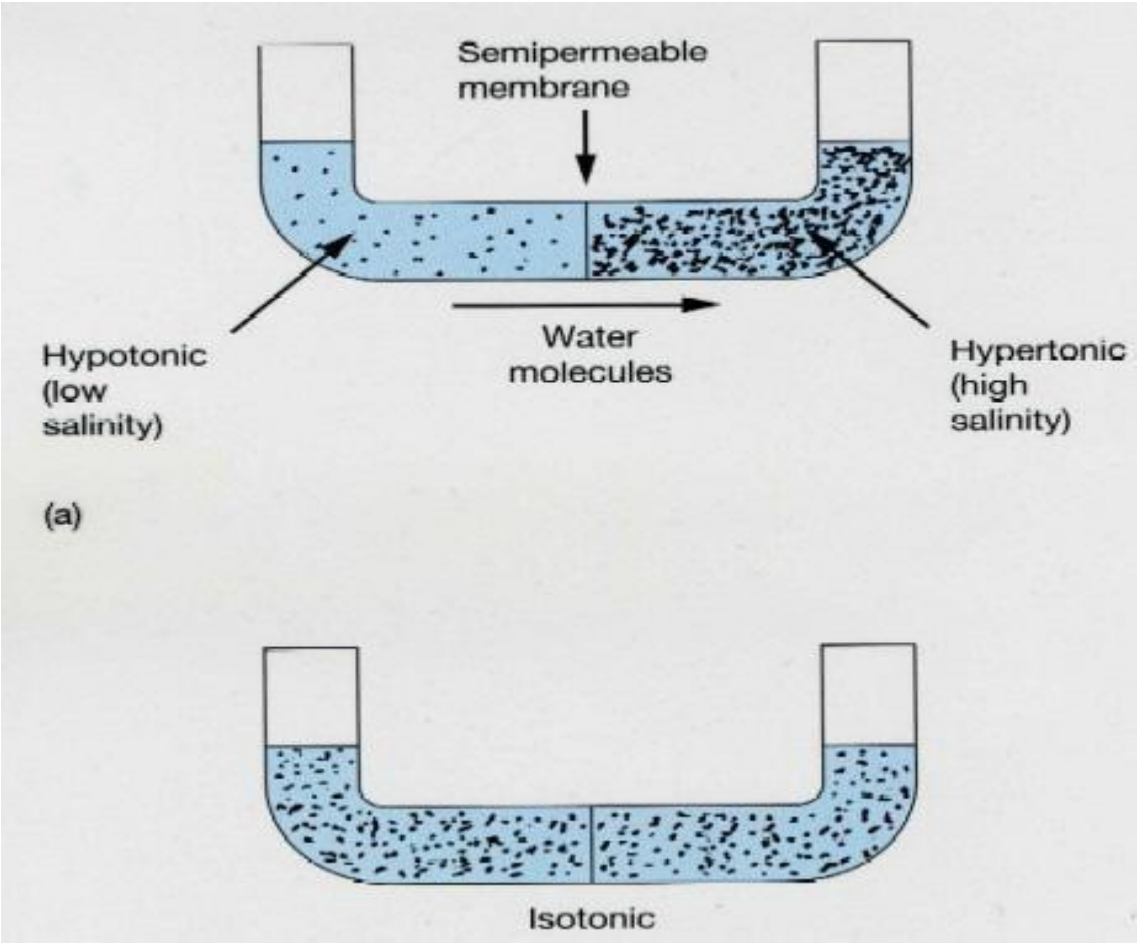
Light - the primary importance of light is photosynthesis, which will be discussed below. The depth of penetration of light will determine the birth of a food chain sequence. This also depends on the light wavelength, and turbidity. Hence most marine organisms live in the well-lighted neritic zone and in the epipelagic zone where food is abundant. Some deep water fish use light for body orientation (even dim light), feeding, and predator avoidance. Some marine organisms produce their own light by biochemical reaction, known as bioluminescence. Organisms typically living at depths within the aphotic zone, (or those that are active at night) such as squids, some fish and shrimps, are bioluminescent. They use light to see, to communicate, and to facilitate predation.

Temperature - the metabolic rate of organisms increases with the temperature of their bodies. A 10 C increase doubles the metabolic rate. This is directly associated with the rate of energy production. Endothermic organismscontrol their own temperature from within, and ectothermic organisms depend on the temperature of the environment. Endotherms are mammals and birds. They can survive in a variety of environments because they can fine-tune their temperature to remain within a narrow range where metabolic rate is optimum. Ectotherms living in warm conditions are more active, have a higher reproduction rate, grow faster, but live shorter lives. Temperature range in the oceans is -50 to 40 C, except around hydrothermal vents where temperatures can be as high as 110 C. So in general, marine organisms live within a much narrower temperature range than land organisms. Temperature range on land is -40 to 50 C.

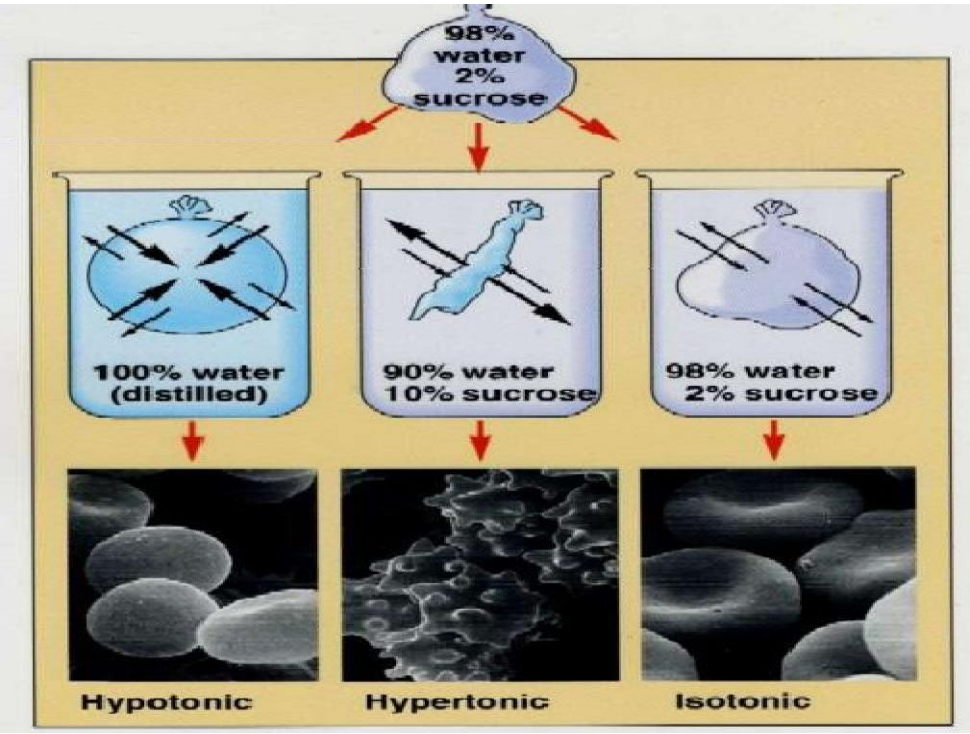
Dissolved Nutrients - Nutrients are chemical substances that play vital role in the growth and general functioning of an organisms. In the oceans, nutrients in short supply are nitrogen (N) and

phosphorus (P), and to a lesser extent Calcium and Silicon (limiting nutrients). Marine plants typically recycle these elements including Fe (iron), Cu (copper), Mg (magnesium), and Zn (zinc).

Salinity - marine salinity varies from 6 - 40 ppt. This large range is controlled by evaporation rates, sea ice formation, and freshwater supply rates. The greatest impact of salinity variation is at the ocean surface, whereas deeper ocean salinity (below the halocline), is far less variable. Salinity affects the tissues of organisms thoroughosmosis.



Most marine organisms are isotonic and no special salinity problems are imposed on them. But marine fish (bony fish) is hypotonic, that is, their body fluids are less salty than seawater. Hence, they are constantly losing water and are threatened by dehydration They overcome this by continuously drinking seawater and expelling the salts through their gills. They also produce highly concentrated urine in very small amounts in order to conserve water. Since salinity affects seawater density, it has an important effect on buoyancy of marine organisms. The average marine fish is denser (1.07) than seawater (1.025) but it can maintain its buoyancy with gas-filled swim bladders. They are constantly adjusting gas volumes as they change depth. Very fast swimmers (and benthic fish) lack swim bladders because they swim so fast that they cannot sink and the benthic ones do not change depths. Planktons store food as oil and have elaborate ornamentations to help them float. Whales and other large marine organisms store low-density fat to increase their buoyancy.



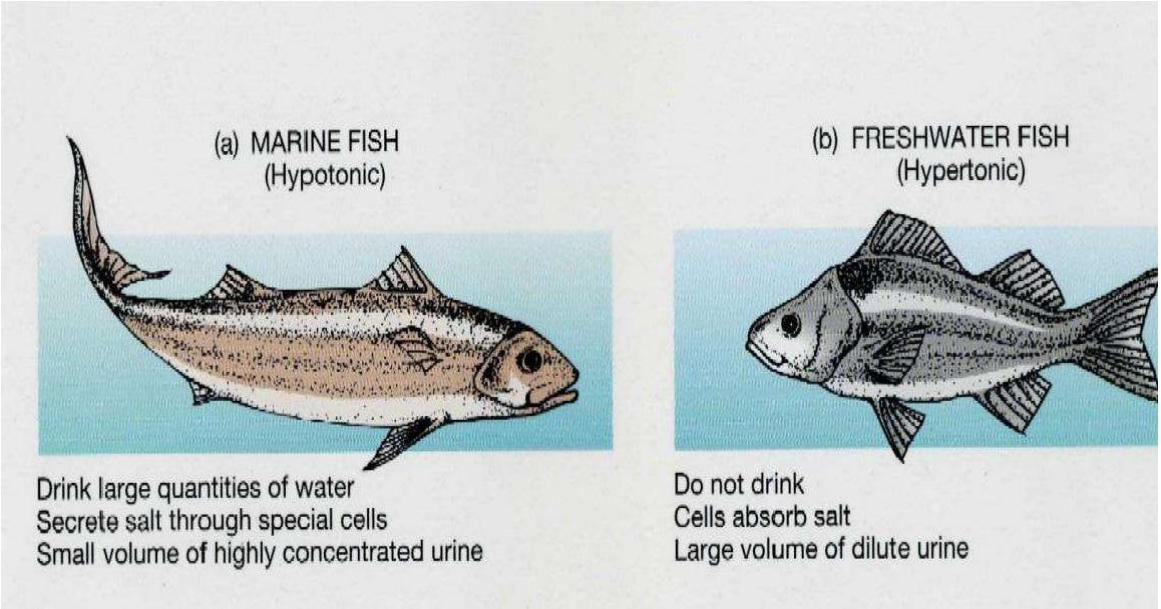
Dissolved gases - gases dissolve more in cold water than in warm water. The two most important gases to marine organisms are: O2, and CO2. O2 is essential for respiration and CO2 for photosynthesis. O2 is less soluble is seawater and tends to be in abundance only in surface waters. Why? CO2 is more soluble in seawater and its concentration increases with depth. Why?

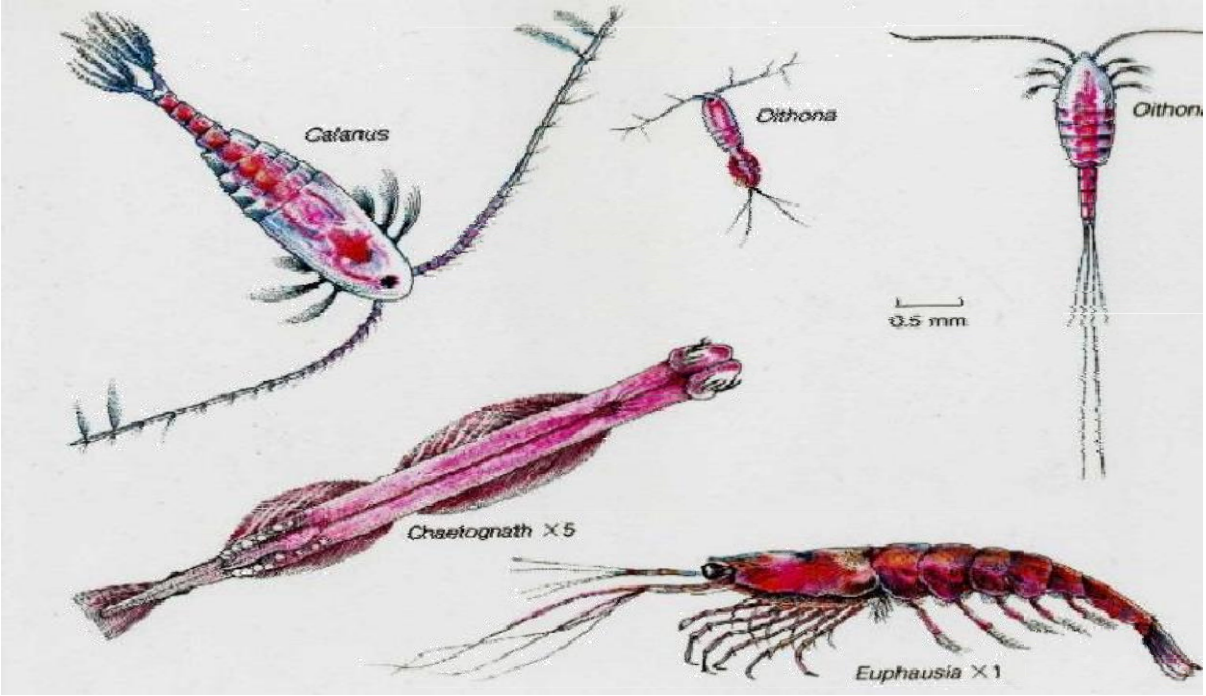
P H - average seawater p H is ~ 8.0, and it is maintained within a narrow range by dissolved CO2

. The calcium carbonate compensation depth (CCD) is the dividing line between more alkaline seawater (8.3), and less alkaline seawater (7.6). The CCD is located between 3,500 m - 6,000 m and averages around 4,500 m depth. Below the CCD, calcium carbonate dissolves, so no CaCO3 shells are formed or survives. Limestone can only be preserved above the CCD. However, terrestrial CO2 pollution is increasing dissolved CO2 concentration in the oceans and the CCD is getting shallower.

Planktons

In the biosphere, nearly all living organisms use converted solar energy as the primary fuel to facilitate their daily activities.

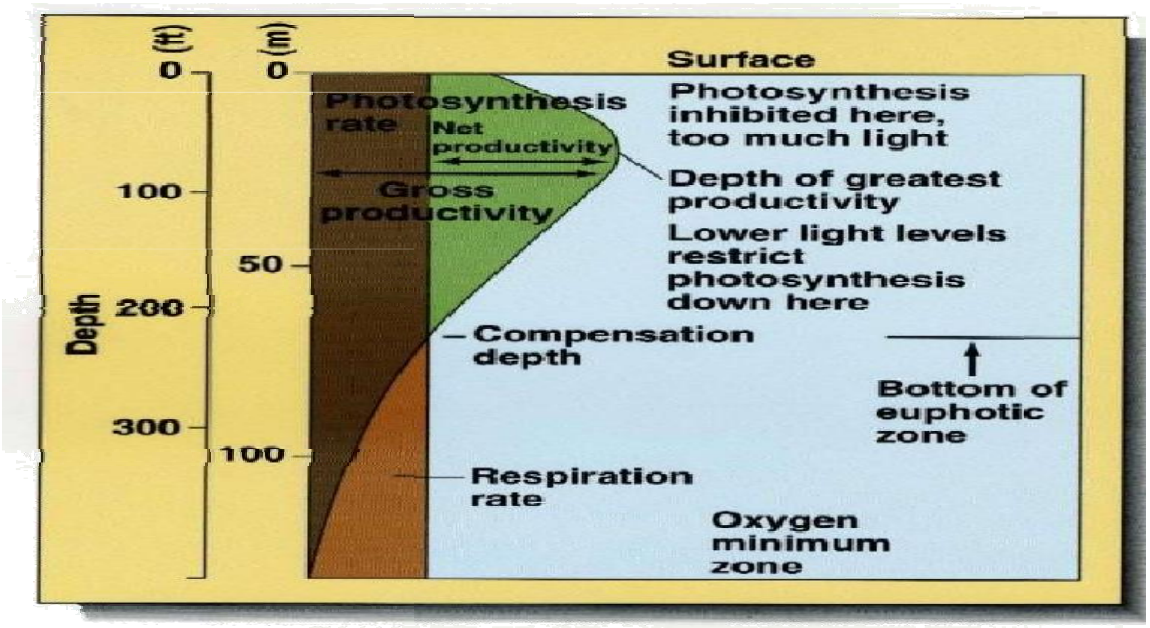




In the oceans, the organisms that capture solar energy and bind it into usable energy for their own use as well for the use of other organisms are known as phytoplanktons and seaweed.

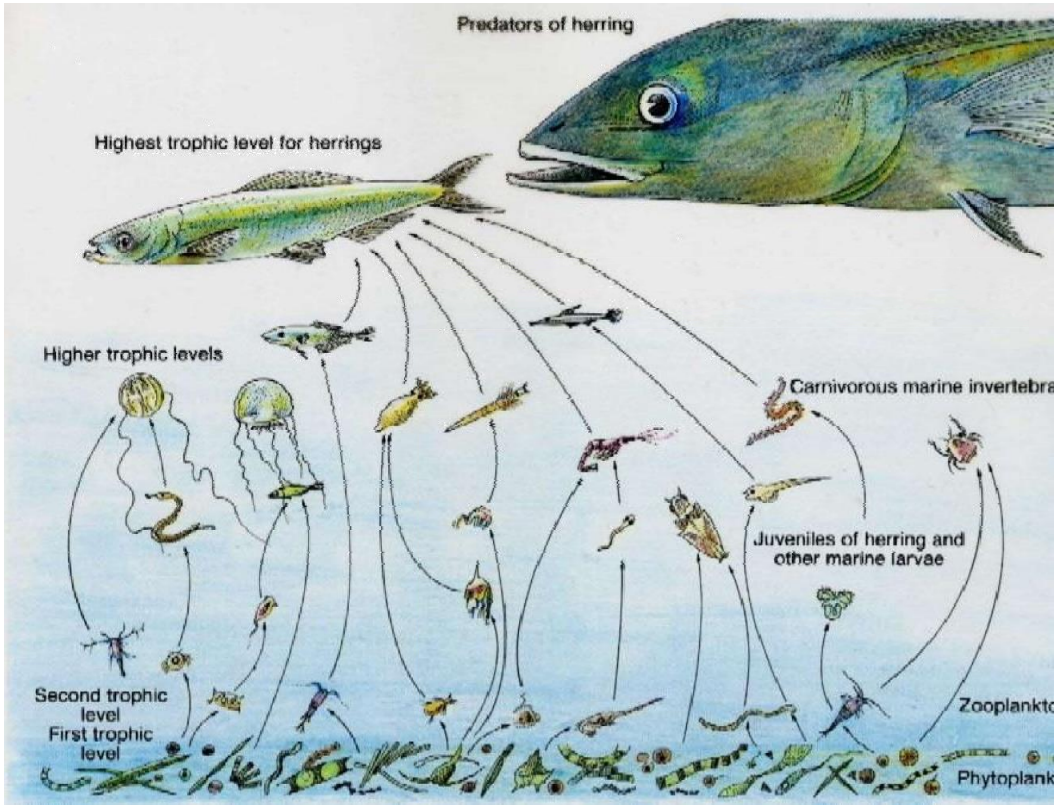
Planktons represent a community of organisms associated solely on their mode of locomotion. All planktons drift or swim very weakly, moving around with the currents or waves. Many can move vertically through the water column. In general, planktons live in the euphotic zone, in the upper layers of the open ocean down to the compensation depth. This is the depth to which 1% of surface light penetrates and photosynthetic organisms produce just enough carbohydrate to

serve all the organisms' needs (zero net productivity). Although the compensation depth is variable, it averages about 150 m from the ocean surface.



Planktons are generally diverse, ranging from those with soft, gelatinous bodies with little or no hard parts, to those encrusted in hard parts. The common planktons are drifting jellyfish, arrowworms, single-celled organisms, some crustaceans, a few marine mollusks, some algae, etc. Hence both animals (zooplanktons) and plants are part of the plankton community.
There are, at least, eight major types of phytoplanktons (the plant variety) responsible for the nearly all the oceans primary productivity. These phytoplanktons are mostly single-celled, microscopic organisms that includediatoms, dinoflagellates, cocclithophores, silicoflagellates, and extremely very minute varieties callednannoplanktons and picoplanktons.

Primary Productivity In oceans, phytoplanktons and seaweed together are known as autotrophs. That is, organisms that make their own food. Such organisms are also known as primary producers. Organisms that do not make their own food but depend on other organisms to provide nutrients are heterotrophs. Heterotrophs obtain a share of the captured solar energy by consuming autotrophs as well as heterotrophs to support their daily activities. Heterotrophs include primary, secondary, and tertiary consumers. Primary consumers are herbivores (plant feeders) like manatees and zooplanktons, that feed directly on autotrophs. Secondary consumers then feed on primary consumers, etc. The natural chain of nutrient and energy interdependency obtained through food consumption or feeding is known as the food web or food chain.



In general, the neritic zone is more productive than the pelagic zone because of constant supply of nutrients and the low-density surface water helps planktons to remain afloat on the water surface longer. This productivity is even higher in areas of coastal upwelling (4 x higher than non-upwelling coastal areas). The open tropical oceans are less productive because of stable thermoclines, but in temperate oceans (40 - 60 degree latitudes), productivity is higher from the seasonal overturn of water masses bringing up nutrient-rich waters. Polar waters are limited by light availability.

MARINE MICROBIAL DIVERSITY

Marine microbes are uniquely important to life as we know it. Since life most likely began in the oceans, marine microor-ganisms are the closest living descendants of the original forms of life. They are also major pillars of the biosphere. Their unique metabolisms allow marine microbes to carry out many steps of the biogeochemical cycles that other organisms are unable to complete. The smooth functioning of these cycles is necessary for life to continue on earth.

Early marine microorganisms also helped create the conditions under which subsequent life developed. More than two billion years ago, the generation of oxygen by photosynthetic marine microorganisms helped shape the chemical environment in which plants, animals, and all other life forms have evolved.

A great deal of research on the biogeography of marine microorganisms has been carried out, but many unknowns per-sist, and more work is needed to elucidate and understand their complexity. It is now known that microorganisms live in every corner of the oceans. Their habitats are diverse and include open water, sediment, bodies of marine macro- and microorganisms, estuaries, and hydrothermal vents. By studying these habitats, scientists have developed a limited ability to predict the composition of marine microbial communities.

It has also been found that some marine microbes have more cosmopolitan distributions than others. Recent work has found that most of the ecological principles that apply to larger organ-isms can also be applied to microorganisms, including marine microbes, but there are exceptions. Almost every ecophysiologi-cal parameter in the oceans is thought to have an impact on the diversity of microbial communities. Most of the direct interactions marine microorganisms have with larger organisms fall into one of two broad categories: symbiosis or pathogenesis. Beneficial microbial symbioses have enabled many invertebrate species to take advantage of habi-tats that would otherwise be unavailable to them. Invertebrates in these relationships may also enjoy the benefits of bioactive

compounds microbes may produce to prevent bio-fouling or to ward off predators. Marine viruses are found in surprisingly high numbers in seawater, but it is likely that these populations are in equilibrium with their host populations.
The exact nature of these impacts cannot yet be predicted. Human health relies on a number of critical equilibria that marine microorganisms broker, including the balance between viruses and their hosts in the oceans, the balances that keep harmful algal blooms in check, the processes that control nutrient concentrations in marine waters, and others.

The metabolic capabilities of marine microbes can be put to work in any number of biotechnology applications, including the manufacture of industrial products and energy production. Marine microbes are sources of novel bioactive compounds that may have application as pharmaceuticals Potential appli-cations for marine microorganisms in ameliorating environ- mental degradation also exist.

Innovative approaches in research, education, and training are critical for moving the field of marine microbiology for-ward. Modern research in this field should embrace the new tools of genomics and metagenomics, but not to the exclu-sion of other methods of discovery. Education and training in marine microbiology needs to be multidisciplinary. Arrangements that expose graduate students and postdoc-toral scientists to laboratories that do work outside the stu-dents' immediate fields of focus should be encouraged.

INTRODUCTION: MARINE MICROBESAND EARTH'SH ABITABILITY

For millions of years after emergence of the first life forms, microbial life in the oceans influenced the planet's chemistry, altering the chemical balance of the oceans and atmosphere and introducing gradients of oxidizing agents (electron-scav-enging) and reducing agents (electron sources).

Early microbes introduced molecular oxygen to the atmosphere, an accomplishment that set the For millions of years after emergence of the first life forms, microbial life in the oceans influenced the planet's chemistry, altering the chemical balance of the oceans and atmosphere and introducing gradients of oxidizing agents (electron-scav-enging) and reducing agents (electron sources).

Early microbes introduced molecular oxygen to the atmosphere, an accomplishment that set the stage for the evolution of plants,animals, and humans.
These microbe-induced changes introduced a new era of chem-istry on the earth—one based primarily on redox chemistry, the shuttling of electrons from one molecule to another. Redox chemistry is now the basis of the balanced biogeochemi-cal and climatological cycles that sustain life on this planet.

Marine microbes also carry out many of the steps in these bio-geochemical cycles, making them the workhorses of the biosphere. The owners of a diverse portfolio of possible activ-ities, marine microbes provide most of the planet's metabolic capabilities that keep elemental cycles in motion. The metabolic rates of marine microbial commu-nities are also high. Although terrestrial organisms comprise the vast majority of the biomass on the planet (3,200 gigatons or more; that is, 10^{15} grams), marine plankton (which weigh in at about 0.4 gigatons) carry out 45% of the total oxygen respiration on earth.

The ocean is filled with microorganisms that dwell there per-manently and other microbes that have been carried there from terrestrial environments. In research on marine microbes, it is often necessary to arrive at a definition of what, exactly, a marine microorganism is. Any of a number of definitions can be used. A simple definition says marine microbes are just that—any microorganisms found in marine systems. However, this description does not exclude organ-isms that wash into the oceans from land and are not suited for growth in the marine environment.

An operational definition of marine microbes describes them as species that can grow and reproduce in the marine habi-tat. The possibility for growth can be determined using iso-tope feeding experiments, in which the organisms in question are monitored for growth on a diet of nutrients like those found in the marine habitat. The problem with this definition is that the reverse may not be true; a lack of growth does not necessarily indicate that a microorganism is not a contributor to the ecosystem. A dormant microbe found in seawater may be biding its time until conditions are right for its growth. A physiological definition identifies marine microbes as pos- sessing adaptations specific to the marine environment. Under this description, marine microorganisms have precise physiological adaptations or even requirements for sodium.

In some cases, the distinction between true residents of the oceans and organisms that wash in from land is unimportant. Sometimes, exotic organisms (or even organisms that die once exposed to the marine environment) can play a role in the ecology of the oceans.

Regardless of the preferred definition, marine microbes hold a position of unique importance in the biosphere. They were the original form of life on earth and today marine microor-ganisms are a primary support for the biogeochemical cycles that continue to make life possible. A great deal of research has been carried out to elucidate the biogeography and metabolism of these organisms, but many unknowns persist. Uppermost on this list of questions is what effects human-induced changes will have on the services marine microbes perform for the planet. Research on marine microbiology must continue or accelerate in order to solve these problems.

MARINE MICROBIAL HABITATS

Marine microbes continue to have a profound influence over the biosphere, but where, precisely, are marine microbes found? What are their various habitats like? How do you aseptically sample the habitats? These questions can be answered in a number of ways, based on the level of resolu-tion that is of interest (see Table 1). The marine environment occurs on many scales, and there are many niche levels from which to approach a description of habitats. For example, a microbe floating in the middle of the Pacific Ocean

could be described as free-living," but this is no more or less accurate than the descriptors "pelagic" (meaning "open water," not sediment) or "within the Central Pacific gyre."

Perhaps the most important factor in defining marine micro-bial habitats is the distance over which these organisms inter-act with their environments. The habitat attributes that are apparent to the naked eye are usually less important to a marine microbe than the microscopic and submicroscopic facts, including concentrations of nutrients, the presence of gels and particulate matter, metal concentrations, light levels, pH, ultraviolet exposure and solar flux, temperature, oxygen saturation, and redox. Hence, the scale at which marine micro-bial habitats are most relevant is very small, but defining the boundaries of these habitats is difficult to accomplish in a con-trolled laboratory experiment and is even more difficult to define for a microbial cell embedded in the environment.

Microbial habitats in the oceans are influenced by an almost innumerable array of forces and factors, including salinity, currents, terrestrial inputs, and climate. Salinity is relatively constant in the open ocean, but is less stable in coastal areas. Ocean and seafloor currents have been shown to behave in ways other than previously thought, greatly affecting our understanding of transport processes in the deep sea. Terrestrial inputs create gradients of nutrients, pollutants, and other matter that affect habitats. Climate effects represent the largest scale of influence on microbial habitats. Temperature, precipitation, and wind (including windborne particulate matter) can each impact marine communities in a number of ways.

HOW THE MARIEN ENVIRONMENT MAY BE DIVIDED INTO DIFFERENT MICROBIAL HABITATS

Criterion	Habitats
Presence f other organisms	Symbiotic
	Free-living
	Biofilm
Proximity to the ocean surface or sediments	Euphotic (0-150 m)
	Mesopelagic (150-1000 m)
	Bathopelagic (>1000 m)
	Benthos (sediments)
Concentration of nutrients and required growth substrates	Oligotrophic
	Mesotrophic
	Eutrophic

Importantly, marine microbes themselves exert influence on their habitats by consuming, producing, and sequestering a variety of compounds. Hence, in the oceans, gradients of materials important to micro- and macroorganisms alike are often controlled by processes carried out by microbes.

In the marine environment and elsewhere, interfaces tend to be hotspots of diversity and biological activity. Marine micro-bial habitats at interfaces include the air-water, water-sedi-ment, water-ice, and host macroorganism- water interfaces. The sub-millimeter scale of physical and chemical variability in these habitats poses a serious challenge to studying inter-face habitats in detail.

CHANGE OVER TIME

Microbial habitats change over many time scales—diel (daily), seasonal, decadal, and longer. Many of the changes induced by human activities can impact marine microbial communities and, in turn, can impact the ways by which those communities modulate the environment and climate. Temporal changes in marine microbial habitats can be illus-trated by describing three disparate habitats: the central Pacific gyre, the Chesapeake Bay, and hydrothermal vents. The central Pacific gyre is an open ocean habitat that changes on a diel basis, but it has also exhibited changes over decades as shifts occur between community domination by diatoms and by picoplankton. The Chesapeake Bay exhibits diel changes, marked seasonal changes, and profound decadal changes over the past couple of centuries as human activities have taken their toll. Hydrothermal vents exhibit both short and long periods of fluctuation, changing over the course of min-utes, hours, and decades. This variability creates an ephemer-al and unpredictable habitat for microorganisms.

In the coming years, if observed trends in greenhouse gas emissions continue, increasing concentrations of atmospheric carbon dioxide are expected to result in a pH decline of 0.3 in the oceans—a small number that signifies big changes. This would be an utterly radical transformation of the ocean habitat for microorganisms and macroorganisms alike. To illustrate, a similar pH shift in the acidity of human blood would result in acidosis and a painful death.

INTERACTIONS BETWEEN SEDIMENT-DWELLING AND PLANKTONIC MARINE MICROBIAL COMMUNITIES

In general, marine microbes in and near sediments interact with and intercept reductants diffusing from the sediments below and oxidants diffusing from the water column above. Conversely, planktonic microbes intercept carbon compounds from photosynthetic activities near the surface of the ocean and control the downward flux of nutrients to the sediments. The connections between subsurface and planktonic environ-ments are

probably greatest in zones where the sea floor is spreading. However, details of the interactions between the microbial communities of marine sediments and communities in the water column are not known—a clear gap in the cur-rent knowledge.

MARINE SEDIMENTS, BIOFILMS, AND EARLY LIFE

In some ways, the physical and chemical circumstances of marine sediments are thought to reflect those that nurtured the beginnings of life on this planet. Studying marine sedi-ment and the life that exists there today could provide insight into early life:

- Redox coupling may have been important to fostering formation of the organic molecules that propagated early life. Marine sediments harbor marked layering of redox potentials that enable extensive redox coupling.

- Methane may have been one of the building blocks of early life, and there are numerous areas of the ocean floor where methane percolates up from the deep subsurface. Heat and pressure could have facilitated formation of early biological molecules, including amino acids. These conditions exist in
- parts of the sea floor that combine high depth with communication with the earth's hot core.

- Given the pivotal role of iron in the functioning of many enzymes, iron is thought to have been abundant where enzyme systems first evolved. Marine sediments are usu-ally iron-rich environments.

- Before the dawn of oxygenic photosynthesis, the earth's atmosphere was anaerobic, a condition mirrored by sub-surface marine sediments.

It can be argued that shallow microbial mats and biofilms are the most appropriate systems for modeling early life, as the high metabolic diversity and spatial separation of metabo-lisms of these arrangements closely reflect fossilized exam-ples of early microbial communities. Microbial communities that dwell in marine sediments can be construed as biofilm or mat communities, given that these communities dwell on the surfaces of sediment particles (like biofilms) and can form thick accumulations of interacting cells (like microbial mats).

COMMUNITY COMPOSITION

Although intuitively apparent, it can be difficult to reach agreement on the precise technical definition of a microbial community. It is largely agreed that microbial communities are groups of microorganisms that interact and, together, accomplish more than those same organisms would sepa-rately. Communities are also influenced by common factors and/or by each other. However, the question of whether chemical interdependence, a form of interaction, is required among members of a community is more controversial. A continuum of interactions, ranging from obligate to minimal, is thought to exist among members of microbial communi-ties; strict interdependence is not necessarily a requirement for the designation “community.” Microbial communities, in which members interact, are distinct from microbial assem-blages, in which members merely coexist.

PREDICTING THE COMPOSITION OF MARINE MICROBIAL COMMUNITIES

The particular physical and chemical conditions of a given marine habitat, including resource availability, select for dis-tinct groups of microorganisms, and there is a certain amount of predictability in the character of the resulting community Mapping the microbial species onto the physical and chemi-cal variability of marine habitats is becoming increasingly fea-sible in certain habitats and with certain well-described species. For example, the general distributions of two plank-tonic genera, *Synechococcus* and *Prochlorococcus*, are well-understood information that

can be extrapolated to unknown planktonic communities. The presence of certain broadly-defined functional groups, such as nitrogen fixers or calcium carbonate producing microbes, can also be predicted.

Most marine microbial communities are not yet fully described, however, so it is difficult, if not impossible, to pre-dict the species-level composition of a marine microbial com-munity. Hence, it is often possible to predict the functions in place in a given marine microbial community, but it is seldom possible to predict the genera or species present.

The scale and level of resolution of the inquiry into a marine microbial community are important factors in predictability. Communities can be unpredictable on a small scale (1 meter) but predictable over larger scales (kilometers). The level of resolution often determines the results of a study on commu-nity diversity. Deep, exhaustive sampling can reveal much greater diversity and complexity than shallow sampling. Predictability in marine microbial communities that is based on 16S rRNA genes may not be corroborated by further work at the genome sequence level. Genome sequences usually reveal much greater and, currently, unpredictable diversity.

The biome concept may be useful in predicting the composi-tion of microbial communities. By this reasoning, the energy inputs into an ecosystem are evaluated, and the role this energy plays in defining the attributes of the microbial com-munity is examined.

Alternatively, a microbial community may be structured by an evolutionary history that prevents the spread of that and establishment of new species into that com-munity. If a community's history dictates its composition, it would be very difficult to predict the struc-ture of the community from transitory features like nutrient status and temperature.

Certain tight associations, like the one between the bacteri-um *Vibrio angulara* and the alga *Ulva*, can allow researchers to use the presence of one species to predict the presence of a partner species. Recent studies have shown *Ulva* propag-ules will not establish themselves on a surface in the

absence of chemical signals from their biofilm partner, *V. angulara*. Hence, if *Ulva* is detected in a biofilm, it can be assumed that *V. angulara*, too, is present.

In seeking to better predict the structure of marine microbial com-munities, there is a need to know more about possible keystone species—organisms that may be present at low or high numbers but perform indispensable functions for the community.

MICROBIAL DISTRIBUTION IN THE OCEANS

The issue of whether marine microbial species are either cos-mopolitan, or are more provincial and limited to certain geo-graphical areas, was raised decades ago. The question endures today because of the bearing it has on the conservation of bio-diversity. If marine microbes are not cosmopolitan, then does the international scientific community need to act to preserve the microbial diversity harbored in endangered habitats?

Current evidence indicates that most marine microbes are not cosmopolitan, but, instead, are restricted to specific habitat types or geographic locations. However, there are a few exam-ples of truly cosmopolitan organisms, including the deep-sea marine group I archaea. As new, higher resolution technologies become available, further research may show other microor-ganisms to be more widespread than previously thought.

Free-living marine microbes may be more cosmopolitan than symbionts, biofilm-associated microbes, and others. Extinction is a real possibility for symbiotic microbes since they are dependent on the survival of their host and many, many species of marine macroorganisms are currently endangered.

It is essential to note that the number of representatives of a given group is not necessarily linked to the importance of that group in the functioning of the community. Common organ-isms may not play a critical role in the dynamics of a given community despite their numbers, and organisms that

only muster 0.1% prevalence, like nitrogen fixers, can be of pivotal importance. Consequently, it is not known whether cosmo-politan microorganisms like *Synechococcus* are common because they are essential to their communities or because they are weedy individualists that can survive in a wide spec-trum of environments.

One observation that appears to support the idea that marine microbes are largely cosmopolitan is the establish-ment of novel microbial communities in the wake of a dis-turbance. Often, the new communities are dominated by microbial types that were previously present in low or unde-tectable numbers.

The long-standing question of how to taxonomically divide microbes creates some confusion in discussions about cos-mopolitan distributions. A working definition of an appropri-ate taxonomic unit is needed to address research in microbial distribution in the oceans and elsewhere.
Millimeter-scale analyses of marine habitats may help to reveal the distribution variability of marine microbes over very small spatial scales.

OUTSTANDING QUESTIONS ABOUT MARINE MICROBIAL DISTRIBUTION

A number of outstanding questions about marine microbial distributions remain to be addressed. These include:

- What is the ecological relevance of the functional gene diversity observed in marine microbial communities? Does the diversity have an impact on ecosystem function or long-term stability?

- Are changes in microbial communities predictive of changes in environmental function?

- The long-term variability of marine microbial communi-ties is poorly understood. Can intermittent sampling, like taking

community snapshots, address this question or is continuous sampling necessary to evaluate community composition shifts?

- Are there keystone species in marine microbial commu-nities that are critical to a given function? How can sci-entists identify those organisms?

FORCES AT WORK IN MAINTAINING STEADY STATE IN MARINE MICROBIAL COMMUNITIES

Many marine surface waters maintain steady populations of approximately 10^6 bacteria and 10^7 viruses per milliliter, a con-dition that may be determined by nutrient limitations and pre-dation controls enforced by protists and viruses. It is thought that, in some cases, microbial population numbers are kept at about an order of magnitude below the carrying capacity of the habitat—presumably by predation. It is thought that viruses may coexist with their hosts, helping to structure communities and diversity, but some studies have shown that the removal of viruses from a system has no effect on bacterial abundance and community structure. Protists, on the other hand, are thought to play a more antagonistic, less discriminating role in steady state maintenance of bacterial cell abundances by consuming many different types of bacterial prey.

MACROECOLOGICAL THEORY AND OCEAN MICROBES

It only became feasible to test ecological theory as it applies to marine microbial communities within the last 10 years, after basic questions about the abundance and species distri-bution of marine microorganisms had been answered. In gen-eral, ecological concepts like predation, competition, and diversity appear to be applicable to marine microbial com-munities, but particular problems related to scale, food webs, the species concept, and the traditional focus on biochemical and geochemical sciences within marine science preclude broad application of ecological theory to these communities.

The small scales relevant to microbes are often not addressed adequately by traditional macroecology. Also, the structure of food webs in microbial

systems is likely to take on a very dif-ferent form than those described by traditional macroecology.

The species concept poses a big stumbling block for apply-ing ecological theory in marine microbial systems. The group-ing of organisms into clusters of species is the basis of most ecological theory, but most microorganisms do not fall neat-ly into species categories because of their ability to repro-duce asexually. It may be that genes or genomes are more appropriate taxonomic units than species for modeling in microbial systems.

To a certain extent, the biogeochemical and geoscience focus of marine science has prevented robust integration of eco-logical and evolutionary theory into marine microbiology. Rigorous inclusion of ecological science within classical oceanographic science is needed to satisfactorily address contemporary challenges in marine microbiology. This will require extensive dialogue between theoretical ecologists, evolutionary scientists, physical and chemical oceanogra-phers, and marine microbiologists.

With man-made pressures on the world's natural systems grow-ing annually, a thorough grasp of the ecology of all environments, the oceans included, will be necessary to mitigate damages and manage our natural resources for the benefit of future genera-tions. Because of their short generation times and relatively sim-ple physiology, microorganisms have been and will continue to be powerful model systems for testing ecological theories.

FACTORS THAT IMPACT MARINE MICROBIAL DIVERSITY

The list of factors that impact marine microbial diversity is not a short one. Nearly every measurable physical chemical, and biotic variable in the marine environment has been found to increase, decrease, or otherwise alter

microbial diversity. See Table 2 for a list of several of the more important factors.
For the most part, the extent to which each of these influ-ences actually operates in the environment and the contexts in which they are important remain to be determined.
Climate change, which will be felt by marine microbial com-munities as changes in ocean temperatures, will undoubtedly alter the diversity of communities in unforeseen ways. Climate change should be considered a major top-down con-troller of microbial communities.

Pollution, including nitrogen inputs due to anthropogenic nitrogen fixation, also impact marine microbial diversity section on Humans and Marine Microbes). Anthropogenic nitrogen inputs to the oceans now comprise about half the total nitrogen inputs to the oceans, a circumstance that has resulted in vast dead zones in coastal areas and an increased incidence of harmful algal blooms.

INTERACTIONS BETWEEN MARINE MICROBES AND MARINE MACROORGANISMS

Most marine ecosystems are fueled by the regeneration of nutrients— processes mediated by marine microorganisms. This is the most fundamental service provided by marine microbes; they are responsible for the cycles that sustain all living things in the oceans.

Microbes also play more direct roles in the health of corals and other marine organisms. For example, corals die when the bacteria that live on their surfaces are removed, although the mechanism behind this observation is not known. Also, bacteria associated with squid eggs have been shown to pro-tect the eggs from fungal infection. Biofilm bacteria are known to broadcast attraction cues that affect the settlement of invertebrate larvae in those biofilms.

S Y M B I O S I S A N D P A T H O G E N E S I S IN THE OCEANS SYMBIOSES WITH INVERTEBRATES

A number of marine invertebrates, including species of corals, sponges, squids, shipworms, and others, are associated with unique species of bacterial and/or archaeal symbionts. Symbiosis between a microbe and a marine invertebrate affords the microbe shelter, nutrients, and possibly a route for reproduction and dispersal, and offers the host a variety of benefits. In some cases, one invertebrate species may be host to many, possibly hundreds, of unique microbial species, a detail that has important implications for marine microbial diversity considering the fact that about 1,000 coral species and more than 5,000 sponge species populate the oceans. The diversity of marine symbionts is an understudied field.

Vertical transmission, passing symbionts from generation to generation to generation through the gemetes, more easily allows the transmission of identical clonal organisms between members of an invertebrate species. The full genome sequences of both vertically and horizontally-transmitted marine symbionts are currently being studied and will probably shed more light on the diversity of these organisms.

Vertical transmission is likely to lead to a close symbiosis between the microbe and its host, since inheriting a symbiont from one;s forbearers affords few opportunities for host and symbiont-switching. In horizontal transmission, for host and symbiont-switching. In horizontal transmission, the host-and symbiont-switching. In horizontal transmission, the host is more likely to acquire symbionts appropriate for the particular location where the host settles down.

The diversity of marine microbial symbionts and the closeness of symbiotic relationships may be determined by the mode of transmission employed by the invertebrate hosts. Transmission is accomplished either by “horizontal” or “ver-tical” means. Studies have found that horizontally-transmit-ted symbionts, which are dispersed in the environment and picked up by invertebrates, are more taxonomical-ly diverse than vertically-transmitted symbionts with respect to the internally transcribed spacer (ITS) regions of their ribosomal DNA. (The ITS region is variable in length and sequence and can be used to identify and establish relatedness between microorganisms.) The reason for this differ-ence may be the fact that It is possible that in the early stages of a new symbiotic rela-tionship, horizontal transmission of a symbiont can be used effectively, but as the

relationship becomes tighter and more necessary to the survival to the microbe and its host, trans-mission must shift to the vertical mode, via the gametes. Symbionts have been known to dispose of those parts of their genomes that are redundant within the protective confines of the host. Giving up part of its genome can render a microbe less fit for survival outside the host, making vertical transmis-sion necessary for the survival of the symbiont.

In studying marine symbionts *in situ*, it can be difficult to sep-arate the symbionts from the pathogens and transient popu-lations of microbes that may also be found in and around an invertebrate. Phylotype-specific probes that preferentially detect microbes with high concentrations of RNA compared to those with lesser concentrations can be used for this purpose.

Symbiosis and the Invasion of New Environments

Symbioses with bacteria and/or archaea have enabled some marine invertebrates to exploit habitats that would otherwise be unavailable to them. Hydrothermal vents, for example, repre-sent an extremely inhospitable environment to the unprepared tube worm, but the tube worms that thrive in these sulfur-rich, oligotrophic zones (low in organic carbon) harbor chemoau-totrophic bacteria that synthesize organic carbon using the energy from respiring reduced inorganic sulfur compounds. The bacteria provide the organic compounds to their hosts, allowing the worms to live on carbon from inorganic sources and open-ing up a new world of habitats for the worms.

Other examples of symbionts that have enabled invertebrates to take advantage of a new environment include nitrogen-fix-ing, cellulose degrading bacterial symbionts, which have allowed their shipworm hosts to live on a diet of wood, and luminescent bacterial symbionts that enable squid to hunt in moonlit waters without casting a shadow that could be detected by predators.

In some cases, obligatory symbioses with microbes can limit the ability of an inverte-brate to invade a new habitat. The temperature limits of the bacterial symbiont *Symbiodinium*, for example, appear to curb the number of loca-tions where the host coral species can survive.

Studies show *Symbiodinium* may also be more sensitive to stress from anthropogenic (human-made) sources than its host, another factor that could limit the habitats where the coral could establish itself.

Symbionts and the Production of Bioactive Compounds

Many highly bioactive compounds have been isolated from marine invertebrates, including a number of materials with biomedical or industrial significance. It is now known that, in many cases, these substances are produced by symbiotic microbes rather than by the invertebrates themselves. Microbial symbionts synthesize many secondary metabolites, e.g., bryozoan and sponge species.

The exact biological function of the bioactive compounds pro-duced by microbial symbionts is not known, but in the case of cytotoxic compounds found in sponges and tunicates (a type of marine worm), they may be used to prevent fouling of the host. Other materials may be used to deter reef fish from feeding on the invertebrates.

Bioactive compounds produced by microbial symbionts may be useful to humans in any of a variety of ways. If the microbes that produce them can be isolated successfully, it may be possible to achieve large-scale production of these materials by industrial fermentation processes similar to those commonly employed by pharmaceutical companies for the large scale production of antibiotics.

MICROBIAL EFFECTS ON THE ECOLOGY AND LIFE HISTORY OF MARINE INVERTEBRATES

Microbial symbionts can have profound effects on their hosts—effects that have consequences for the ecology and life history of these invertebrates. For example, local microbial diversity has been shown to impact the suite of symbionts harbored by a widespread species of mussel. Studies of the ITS region of the ribosomal DNA of mussel symbionts and their environments have shown that one species of mussel has different

populations of bacteria in diff-erent geographic areas, a reflection of the bacterial populations avail-able for colonization.

In more rigid, obligatory symbioses, like that between the hydrothermal vent tube worm *Riftia* and its symbionts, the presence of free-living symbionts is necessary for an invertebrate to colonize a given area, pos-ing a limitation on the habitats available to that organism. In another example of a tight relationship (as described previ-ously), the bacterium *Vibrio angulara* is required for the algae *Ulva* to establish itself on a surface.

There is some evidence that the algae to blame for harmful algal blooms (which have caused as much as $1 billion dam-age over the past decade) require not only the appropriate nutrients in order to proliferate into a bloom, but they are also reliant on certain bacterial associates. In other words, if the bacterium is present, an algae bloom is possible and if the bacterium is missing then a bloom may not happen. The mechanism behind this phenomenon is not yet known.

SCALE OF MICROBIAL PROCESSES IN THE SEA

Microbial interactions and processes have implications over a very wide range of scales in the oceans, from the nanometer scale (0.0000000001 meter) to the kilometer scale (1,000 meters) and greater. The global outcome of microbial meta-bolic processes is the integration of interactions on very small scales. In designing studies of marine microbial communities, it is important to remember the importance of microscales. They should not be overlooked.

GLOBAL CYCLES OF BIOELEMENTS

The cycles of nitrogen, oxygen, carbon, sulfur, phosphorous, iron, and other bioelements that sustain life on this planet are driven, in part, by the microorganisms in the oceans. Microbes are capable of using every natural compound on the planet and most of the human-made compounds as well. It is this metabolic flexibility that secures microbes' importance in the cycling of the bioelements. Microorganisms control the rate-limiting steps of the cycles that no other organisms can exe-cute. Microbes also strengthen the feedback systems that increase the stability of the cycles of

the bioelements. The details of many key processes in the bioelemental cycles remain unknown, and it is largely unknown which microbes are the largest contributors to these cycles, or if it is even pos-sible for one species to be dominant.

Although the exact contributions of marine microbes to the biogeochemical cycles is uncertain, because of their metabol-ic capabilities and their sheer numbers, marine microorgan-isms are thought to be major players in every cycle relevant to life. It is estimated that if the oceans were emptied of microbes, the carbon dioxide in earth's atmosphere would increase sevenfold. Moreover, half of the microbially-mediat-ed nitrogen fixation occurs in the oceans, and nitrification and denitrification, two key processes that set the pace of the nitrogen cycle, are carried out by microbes. Marine microbes may also play a role in cloud formation by cycling compounds such as dimethylsulfide into the atmosphere. A constant efflux of methane from the surface of the oceans has been detected, and although the process is not entirely under-stood, it is undoubtedly microbially-mediated.

As more and more of the key players in the cycles of bioele-ments are cultivated and studied in the laboratory, and as metagenomic studies continue to contribute to the map-ping of metabolic processes onto ocean depths and provinces, scientists are coming to a better, more sophisti-cated understanding of how elemental cycling is carried out in the oceans.

UNIQUE METABOLIC CAPABILITIES OF MARINE MICROBES

Marine microorganisms possess a number of metabolic capa-bilities that cannot be found in terrestrial microorganisms. As in other ecosystems, the geochemical habitat drives the evolution of different metabolic capabilities in the marine environ-ment. For example, cold environments near the arctic drive different adaptations than high temperature, high pres-sure habitats near hydrothermal vents. Methane seeps are another example of a uniquely marine environment that has motivated novel metabolic capabilities. In methane seeps, high sulfate concentrations combine with high methane

concentrations to favor the anaerobic oxidation of methane, a metabolism found only in the oceans.

The light-driven proton pump proteorhodopsin, which is found only in marine bacteria, is thought to play an important role in the energy balance of the biosphere because of its ability to efficiently generate energy from light. The use of sodium-dependent transporters is also limited to marine microorganisms.
Marine symbioses, including the symbioses between macroorganisms and bioluminescent bacteria and between shipworms and nitro-gen-fixing cellulolytic bacteria, have given rise to many unique metabolic activities.

ADAPTING TO EXTREME ENVIRONMENTS

The oceans are host to many different kinds of extreme environments, and marine microbes have found numerous ways to thrive in those places by either changing their biochemistry to cope with the conditions or by creating barriers to keep the harsh conditions out of their cells.

MARINE VIRUSES

At first glance, the numbers of viruses found in marine waters appears to be exceedingly high. Locations studied to date have revealed viral counts on the order of 10,000,000 virus-es per milliliter of water. However, these numbers are less sur-prising when one considers the number of prospective micro-bial hosts available to those viruses. Approximately 1,000,000 microbes are found in a milliliter of seawater, making the ratio of viruses to hosts roughly 10:1, a reason-able proportion for ensuring that a virus meets up with prospective hosts often enough to propagate itself before is disintegrates. The half life of viruses averages between two to four days, so a virus population must be sufficiently large to ensure that at least some of its members meet up with an appropriate host during that window of time. Viruses that infect rare hosts may need to live for longer periods of time to survive the interval between infection events.

Marine viruses likely play a number of important roles in the ecology of marine microbes. Obviously, viruses act as preda-tors, causing the mortality

of marine microbes. Viruses also help to maintain the high levels of microbial genetic diversity observed in marine ecosystems because hosts are known to manipulate their genomes to evade diseases. By moving DNA between the host cells, viruses act as agents of sex, shuffling genetic information around the community and providing new and surprising combinations of genes in their hosts.

Viruses can also play a role in the ecology of host cells by lysogenic conversion, a phenomenon in which a phage changes the phenotype of a host cell by either introducing genetic material into the host's genome or by other means. It has been found, for example, that marine viruses can carry the genes necessary for photosynthesis, and that these genes are regularly transferred between host cells of *Prochlorococcus*. This temporary storage in viruses and the efficient shuffling of the genes among *Prochlorococcus* species probably has had a profound impact on the evolution of these photosynthesis genes and on the ecology of *Prochlorococcus*.

The diversity of marine viruses is not limited to bacterio-phages (viruses that infect and lyse bacteria). Many other forms of viruses, including DNA and RNA viruses with a wide variety of different sizes, host ranges, and biological proper-ties, remain almost entirely uncharacterized. Marine viruses represent a new, unexplored world of diversity.

THE ABUNDANCE AND DISTRIBUTION OF BACTERIAL AND VIRAL PATHOGENS

In the marine environment, as elsewhere, the distribution of a bacterial or viral pathogen is directly determined by the viru-lence of the pathogen and the number of susceptible hosts available. This balance between hosts and pathogens generates and maintains the diversity of both groups. However, this deli-cate relationship breaks down in some instances. For example:

- Lateral gene transfer between bacteria (carried out by viruses) and lysogenic conversion may be important
- mediators of change from non-pathogenic to pathogen-ic states in some bacteria.

- Opportunistic pathogens like *Vibrio cholerae* do not comply with the rules of host availability since they can exist outside the host.

- Climate-related factors, including temperature, have been shown to trigger a pathogenic state in certain opportunistic pathogens like *Vibrio shiloi* and coral sym-biotic algae called zoozanthellae.

- Human activities and chemical pollution can also influ-ence the abundance of pathogens by stressing and destabilizing microbial communities.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.Tech. BIOTECHNOLOGY

UNIT – II - MARINE BIOTECHNOLOGY – SBT1304

UNIT II: Aquaculture and Fish genetics (12 hrs)

Aquaculture: Definition- Criteria of selection of aquaculture species. Culture practices of marine fish, shrimp, crab, lobster, edible oyster, pearl oyster and seaweeds. Fish genetics: Gynogenesis, androgensis, polyploidy, artificial insemination, eye stalk ablation-cryopreservation.

AQUACULTURE

CRITERIA OF SELECTION OF CANDIDATE SPECIES FOR AQUACULTURE.

The choice of culture species is, in more ways than one, closely linked with the objectives of the development and therefore the strategy/approach to be used to achieve set goals. Not all fish species are suitable for aquaculture. By the same token, some cultivable species are more appropriate for large-scale, commercial aquaculture rather than for small-scale operations, as exemplified by the high-value shrimps, the production of which can hardly be undertaken profitably on a small scale. Also, some species are best cultured using specific types of enclosures; for example, penaeid shrimps are best cultured in fish ponds rather than in fish pens, and certain species are more acceptable in certain countries than in others.

The choice of species for culture depends on a number of factors including the availability of suitable sites for culture, the biological characteristics of the indigenous or introduced/exotic species, their suitability for culture, and their acceptability in the local or international markets, and the availability of technology and other requirements for their culture.

Principal aquaculture species in Asia

Common Name	Scientific Name	Culture System*	Environment**
FINFISHES			
Milkfish	Chanos chanos	E, S, I	F, B, S
Freshwater eel	Anguilla japonica	EX, E, I	F
	Anguilla spp.		
Grey mullet	Mugil cephalus	EX, E, I	F, B, S
Cockup	Lates calcarifer	EX	F
Grouper	Epinephelus spp.	EX	S

Porgy	Mylio macrocephalus	EX	S
	Mylio spp.		
Red porgy	Chrysophry major	S, I	S
Black porgy	Acanthopagrus schlegeli	S	B, S
Tilapia	Oreochromis mossambicus	SI	F. S
	O. nilotica	E, SI	F, S
	Tilapia zillii	S	F
	O. aureus	S	F
	O. mossambicus x O. niloticus	S	F
	O. niloticus x O. aureus	S	F
Red tilapia	Oreochromis spp.	S, I	F, B, S
Sweet fish, ayu	Plecoglossus altivelis	I	F
Common carp	Cyprinus carpio	E, S	F
Goldfish (wild)	Carassius auratus	E, S	F
Crucian carp	Carassius carassius	E, S	F
Puntius carp	Puntius gonionotus	E, S	F
	Puntius spp.		
Rohu	Labeo rohita	EX, S	F
Mrigal	Cirrhina mrigala	EX, S	F
Bottom carp	Cirrhina molitorella	E, S	F
Catla	Catla catla	EX, S	F
Grass carp	Ctenopharyngodon idellus	E, S	F
Black or snail carp	Mylopharyngodon piceus	E, S	F
Silver carp	Hypophthalmichthys molitrix	EX, E, S	F
Bighead carp	Aristichthys nobilis	EX, E, S	F
Nilem	Osteochilus hasselti	EX, E	F
Walking catfish	Clarias batrachus	E, S	F
	Clarias spp.		
MOLLUSCS			
Japanese oyster	Crassostrea gigas	E, I	S
Hard clam	Metrix lusoria	I	S
Small abalone	Haliotis diversicolor	I	S
Corbiculas	Corbicula fluminea	E	F
	C. Formosa	E	F
Purple clam	Soletellina diphos	E	S

Apple snail	Ampullarius insularum	S, I	F
Blood clam	Tegillarca granosa	S	S
	Crassostrea malabonensis	E	S
	C. iredalei	EX, E	S
	C. palmipes	S	S
	C. cuculata	EX, S	S
	C. lugubris	E	S
	C. belcheri	E	S
	C. commercialis	S	S
	Metrix metrix	EX, S	S
Cockle	Andara granos	E, S	S
Green sea mussel	Mytilus smaragdinus	EX, E, S	S
REPTILES			
Soft-shell turtle	Trionyx sinensis	I	F
Crocodile	Crocodilus siamensis	I	F
	C. porocus	I	F
AMPHIBIANS			
Bull frog	Rana catasbiana	S	F
Tiger frog	Rana tigrina	I	F
SEAWEEDS			
Gracilaria	Gracilaria spp.	E	B, S
Nori	Porphyra spp.	E	S
Wakame	Undaria pinnatifida	E	S
Green laver	Monostroma nitidum	E	S

*EX = experimental, E = extensive, S = semi-intensive, I = intensive

**F = freshwater, B = brackish water, S = saltwater

Source: Liao, 1988

Huet and Timmermans (1972) list the following criteria for evaluating the suitability of a species for culture:

(i) It must withstand the climate of the region in which it will be raised. Thus, the rearing of coldwater fish like salmonids and trout is limited to temperate regions or mountain areas of tropical countries because they can not tolerate warm water with its low oxygen content.

(ii) Its rate of growth must be sufficiently high. Small species, even if they reproduce well in ponds and accept formulated diets, are not the most suitable for rearing. Also, the best culture

species are those which are low in the food chain, e.g., plankton feeders, herbivores, and detritivores. Their culture is also least expensive, even on an intensive scale, because they do not need to be given diets which have a high content of animal protein.

(iii) It must be able to reproduce successfully under culture conditions. Species for culture should be able to reproduce in captivity/confinement without needing special conditions that have to be fulfilled, and which give high returns on eggs and fry. Although it is possible to rear species whose reproduction in confinement is not possible at all (e.g., some carps) or whose reproduction under hatchery conditions has not yet been possible on a commercial scale (e.g., milkfish in the Philippines), the sustainability of the grow-out operations is hampered by the seasonal unavailability of wild fry for stocking in fish pens and/or fish ponds.

(iv) It must accept and thrive on abundant and cheap artificial food. Culture species which feed on cheap artificial feeds and give low feed conversion ratios (FCRs), also tend to give very good production rates, thus bringing in better financial returns.

(v) It must be acceptable to the consumer. Even if all the foregoing criteria are met by a candidate species, it is not worth culturing if there is no market for it. It is possible, though, to promote acceptability of or encourage consumption of a particular species to ensure that it will eventually sell in the market. (This was the situation with tilapia in the Philippines prior to the introduction of the bigger-sized, lighter coloured S. niloticus in the early 1970s.)

(vi) It should support a high population density in ponds. Social and gregarious species which can grow well to marketable size even under high density conditions in ponds or tanks (e.g., tilapia) are preferable to those which can be grown together in dense numbers only up to a certain age beyond which they eat each other (e.g., pike).

(vii) It must be disease-resistant. Reared fish must be resistant to disease and accept handling and transport without much difficulty. Tilapia is an ideal species for culture because of its high resistance to disease even in highly intensive culture systems.

A wide variety of fish and aquatic resources is cultured in freshwater, brackishwater, and marine environments world-wide using different methods (Table 4). Rabanal (1988a) estimates that there are close to 50 species of freshwater, brackishwater, and marine finfish species; about 13 crustacean species, 13 molluscan species, 5 seaweed species, and 5 economic aquatic vertebrates (frogs and other amphibians and turtles and other reptiles) cultivated in Southeast Asia.

Liao (1988) lists some 25 major finfish species, 18 molluscan species, 2 reptile species, 2 amphibian species, and 4 seaweed species as the principal species cultured in Asia. To the list could be added the crustaceans consisting of the brackishwater/marine penaeid shrimps (mainly Penaeus monodon, P. semisulcatus, P. japonicus, P. orientalis, P. merguiensis, and Metapenaeus ensis) and the freshwater prawn of the genus Macrobrachium; the seaweeds Eucheuma, Laminaria, and Porphyra; and marine finfishes like sea bass and groupers (Baluyut, 1989a).

Examples of aquaculture practices employed in different countries for different species

Country	Species Raised	Mode of Culture	Reference
NEPAL	Common carp, Chinese carp, Indian carp	Integrated fish farming	Pullin, 1989
THAILAND	Penaeid shrimps, freshwater prawn (Macrobrachium)	Pond and cage culture in freshwater and brackishwater	Sirikul, et al., 1988
	Cockles and mussels	Mariculture along the coast	
	Finfishes	Cages suspended in rivers and standing waters	
	Clarias batrachus		
	C. macrocephalus		
	Tricnogaster pectoralis		
	Pangasius sp.		
	Lates calcalifer (sea bass)		
	Epinephelus spp. (grouper)		
INDIA	Indian carps	Integrated rice-fish culture	Mukhopadhyay, 1989
YUGOSLAVIA	Primary species:	Pond culture	Wurtz, I960
	Table carp		
	Aischgrund sp.		
	Croatian sp.		
	Secondary species:		
	European catfish		
	Tench		
	Pike		
POLAND	Carp, tench, pike	Pond culture	Ackefors, 1989
EAST GERMANY	Carp, tench	Pond culture	Ackefors, 1989
ECUADOR	Penaeid shrimps	Pond culture	ADCP, 1989b
EL SALVADOR	T. aurea	Pen culture; floating cage culture	FAO, 1986
	T. mossambica		
GUATEMALA	Tilapia and carp	Cage and pond culture	FAO, 1986
COSTA RICA	Tilapia	Pond and cage culture	FAO, 1986
	Trout	Semi-intensive pond culture	
	Chinese carp		

	Freshwater shrimp		
	Crayfish		
	Giant clam		
	(Anodontis luteola)		
ECUADOR	Trout	Intensive pond and cage culture	FAO, 1986
	Trout, marine shrimp	Extensive culture	
AUSTRALIA	Salmonids, marine shrimps, molluscs	Intensive/semi-intensive pond culture	Nelson, 1988
FRENCH			
POLYNESIA			
GUAM	Giant clam, seaweeds, and pearl oysters	Open water culture	
NEW CALEDONIA			
NEW ZEALAND	Tilapia, milkfish, catfish, freshwater prawns, and crayfish	Less intensive onshore pond farming	

In Africa, the predominant species are the tilapias, carps, mullets, sea bass, and catfishes; in addition, some salmonids, miscellaneous freshwater fish, molluscs, and crustaceans are also cultured. Latin America grows miscellaneous exotic fish and marine shrimps, molluscs, and salmonids. Successful experiments on the artificial reproduction and pond culture of indigenous finfishes of the genus Colossoma and Piaractus (locally known as "tambaqui" and "pirapitinga" in Brazil, "cachama" and "morocoto" in Venezuela, "gamitama" and "parco" in Peru, and "cachama negra" and "cachama blanco" in Colombia) also give promise of increased yields (Saint-Paul, 1989). The Caribbean rears tilapia, carp, marine and freshwater crustaceans, oysters, and seaweeds; in the Mediterranean region, salmonids are the prime fish and carps are secondary fish. In the Pacific, tilapia, milkfish, catfish, salmonids, marine and freshwater crustaceans, molluscs (including giant clams and pearl oysters), and seaweeds are cultured but mostly on a pilot/experimental scale (ADCP, 1989a).

FISH CULTURE (SEABASS CULTURE)

Introduction

Lates calcarifer (Bloch), commonly called the giant sea perch or seabass, is an economically important food fish in the tropical and subtropical regions of Asia and the Pacific. It is commercially cultivated in Thailand, Malaysia, Singapore, Indonesia, Hong Kong and Taiwan, in both brackishwater and freshwater ponds, as well as in cages in coastal waters. Because of its

relatively high market value, it has become an attractive commodity of both large to small-scale aquaculture enterprises. However, the major constraint to rapid expansion of seabass culture has been the inconsistent supply of fry collected from the wild. It fluctuates widely from year to year, making forward planning for production difficult.

In the early 1970's, Thai scientists have achieved success in the breeding of seabass under captive conditions. Completion of its life cycle has also been accomplished. Growth performance of the hatchery-bred fry has been shown to be comparable with that of fry collected from the wild. Thailand is presently producing more than 100 million fry annually (Anon. 1985), with the Satul Fisheries Station producing more than 30 million (Kungvankij 1984). Thus, the seabass culture industry in Thailand is now assured of sufficient and consistent supply of fry.

In order to extend the technology of seabass culture, this manual is prepared to serve as a practical guide for extension workers and farmers. Its contents are based on research findings in addition to many years of accumulated practical experience and field observations.

1. Taxonomy

Phylum	Chordata
Sub-phylum	Vertebrata
Class	Pisces
Sub-class	Teleostomi
Order	Percomorphi
Family	Centropomidae
Genus	Lates
Species	Lates calcarifer (Bloch)

The above is an accepted taxonomic classification of seabass or giant perch. Seabass has been placed under several families by various authors in the past (e.g. the grouper family, Serranidae and family Latidae, etc.) However, Centropomidae is the commonly accepted familya name of this species, and the recognized generic name is Lates. Other names such as Perca, Pseudolates, Holocantrus, Coins, Plectropoma,Latris, and Pleotopomus were also given by various authors who collected the fish specimens from different areas. Bloch (Schneider 1801) stated that Lates calcarifer occured in Japan Sea but named it as Holocentrus calcarifer.

The common local names of this species are listed below:

Common English name	: Giant perch, white seabass, silver seaperch, giant perch, palmer, cock-up seabass
India	: Begti, bekti, dangara, voliji, fitadar, todah
East Bengal	: Kora, baor
Sri Lanka	: Modha koliya, keduwa
Thailand	: Pla kapong kao, pla kapong
Malaysia	: Saikap, kakap
North Borneo	: Ikan, salung-sung
Vietnam	: Ca-chem, cavuot
Kampuchea	: Tvey spong
Philippines	: Kakap, apahap, bulgan, salongsong, katuyot, matang pusa
Indonesia	: Kakap, pelak, petcham, telap
Australia and Papua New Guinea	: Barramundi

2. Morphology and distinctive characters (after FAO 1974)

Body elongated, compressed, with deep caudal peduncle. Head pointed, with concave dorsal profile becoming convex in front of dorsal fin. Mouth large, slightly oblique, upper jaw reaching to behind eye; teeth villiform, no canine teeth present. Lower edge of preoperculum with strong spine; operculum with a small spine and with a serrated flap above original of lateral line. Dorsal fin with 7 to 9 spines and 10 to 11 soft rays; a very deep notch almost dividing spiny from soft part of fin; pectoral fin short and rounded; several short, strong serrations above its base; dorsal and anal fins both have scaly sheath. Anal fin round, with three spines and 7–8 soft rays; caudal fin rounded. Scale large ctenoid (rough to touch).

Colour: two phases, either olive brown above with silver sides and belly in marine environment and golden brown in freshwater environment (usually juveniles). Blue-green or greyish above and silver below (adult).

3. Distribution

3.1 Geographic distribution

Seabass is widely distributed in tropical and sub-tropical areas of the Western Pacific and Indian Ocean, between longitude 50°E - 160°W latitude 24°N – 25°S **(Fig. 1).** It occurs throughout the northern part of Asia, southward to Queensland (Australia), westward to East Africa (FAO 1974).

3.2 Ecological distribution

Seabass is a euryhaline and catadromous species. Sexually mature fish are found in the river mouths, lakes (e.g. Songkhla lake) or lagoons where the salinity and depth range between 30–32 ppt and 10–15m, respectively. The newly-hatched larvae (15–20 days old or 0.4–0.7cm) are distributed along the coastline of brackishwater estuaries while the 1-cm size larvae can be found in freshwater bodies e.g. rice fields, lakes, etc. (Bhatia and Kungvankij 1971). Under natural condition, seabass grows in freshwate and migrates to more saline water for spawning.

4. Life history

Seabass spends most of its growing period (2–3 years) in freshwater bodies such as rivers and lakes which are connected to the sea. It has a rapid growth rate, often attaining a size of 3–5 kg within 2–3 years. Adult fish (3–4 years) migrate towards the mouth of the river from inland waters into the sea where the salinity ranges between 30–32 ppt for gonadal maturation and subsequent spawning. The fish spawns according to the lunar cycle (usually at the onset of the new moon or the full moon) during late evening (1800–2000 hours) usually in synchrony with the incoming tide. This allows the eggs and the hatchlings to drift into estuaries. Here, larval development takes place after which they migrate further upstream to grow. At present, it is not known whether the spent fish migrates upstream or spends the rest of its life in the marine environment **(Fig. 2).**

Smith (1965) noted that some fish spend their whole life in freshwater environment where they grow to a length of 65 cm and 19.8 kg body weight. The gonads of such fish are usually undeveloped. In the marine environment, seabass attaining a length of 1.7 m have been recorded in the Indo-Australian region (Weber and Beaufort 1936).

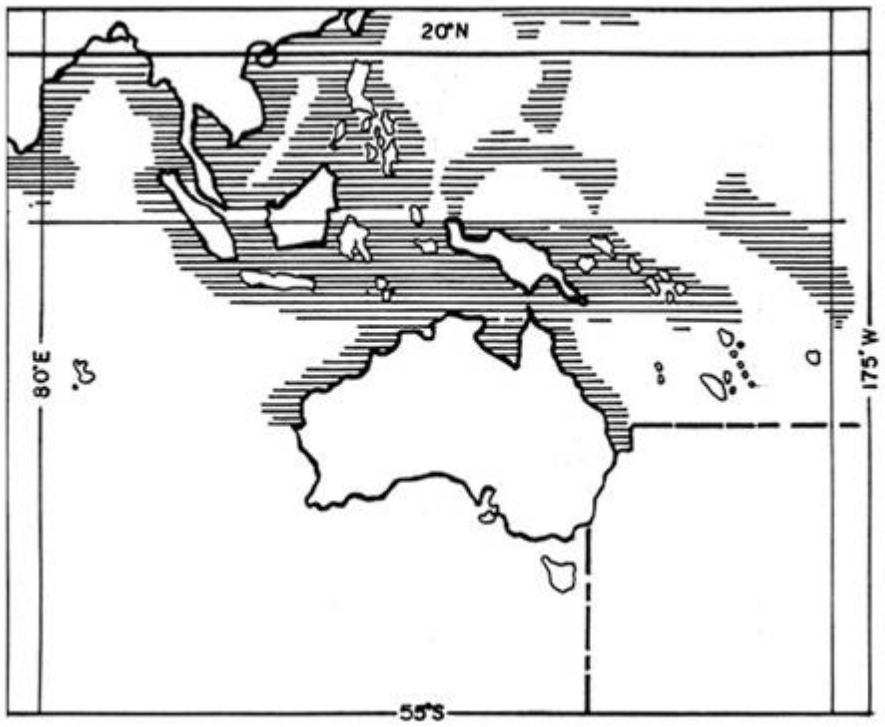


Fig. 1. Geographic distribution of Lates calcarifer. (After FAO 1974)

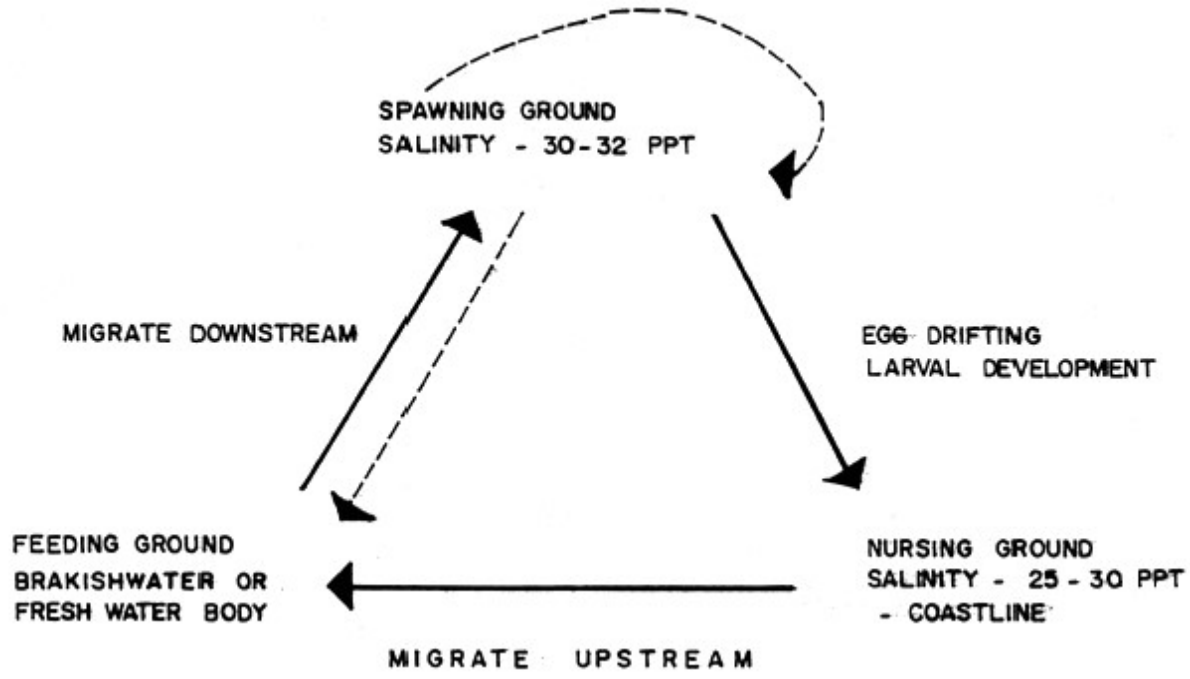


Fig. 2 Migration pattern of Lates calcarifer Bloch

5. Feeding habits

Although the adult seabass is regarded as a voracious carnivore, juveniles are omnivores. Analysis of stomach content of wild specimens (1–10 cm) show that about 20% consists plankton, primarily diatom and algae and the rest are made up to small shrimp, fish, etc. (Kungvankij 1971). Fish of more than 20 cm, the stomach content consists of 100% animal prey: 70% crustaceans (such as shrimp and small crab) and 30% small fishes. The fish species found in the guts at this stage are mainly slipmouths or or pony fish (Leiognatus sp.) and mullets (Mugil sp).

6. Sex determination

Identification of the sexes is difficult except during the spawning season. There are some dimorphic characters that are indicative of sex **(Fig. 3).**

- Snout of the make fish can be slightly curved while that of the female is atraight.
- The male has a more slender body than the female.
- Weight of the female is heavier than males of the same size.
- The scales near the cloaca of the males are thickers than the female during the spawning season.
- During the spawning season, abdomen of the female is relatively more bulging than the males.

7. Sexual maturity

In the early life stages (1.5–2.5 kg body weight) majority of the seabass appear to be male but when they attain a body weight of 4–6 kg majority become female. After culture period of 3–4 years, however, in the same age group of seabass both sexes can be found and identified as mentioned above. In a fully mature female, the diameter of the oocysts usually range from 0.4 ro 0.5 mm.

8. Fecundity and spawning

The fecundity of seabass is related to the size and weight of the fish. Gonad samples obtained from 18 females of body weight ranging from 5.5 to 11 kg gave a range of 2.1 to 7.1 million eggs (Wongsomnuk and Maneewongsa 1976) as illustrated in **Table 1.** Observations by the Australian Department of Agriculture (Anon. 1975) showed that a 12 kg fish had 7.5 million eggs; a 19 kg fish 8.5 million and a 22 kg. fish, 17 million.

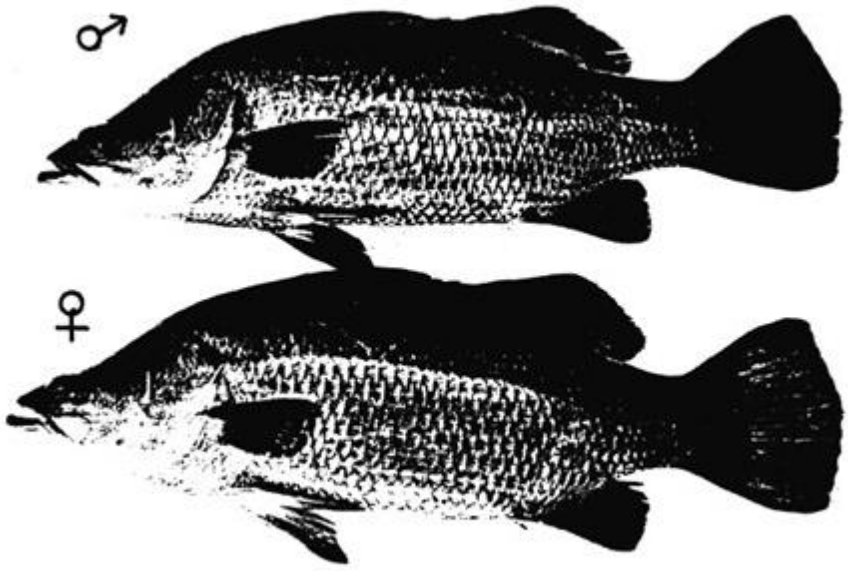


Figure 3 Photograph of adult male and female seabass

Table 1. Relationship between size of fish and number of eggs from the gonads of seabass (Lates calcarifer Bloch). (After Wongsomnuk and Maneewongsa 1976)

Total length (cm)	Weight	Fecundity (million eggs) Average
70 – 75	5.5	3.1
76 – 80	8.1	3.2
81 – 85	9.1	7.2

86 – 90	10.5	8.1
91 – 95	11.0	5.9

Seabass spawn all year round (Kungvankij 1984) with the peak season occuring during April-August and large number of fry (1 cm in size) can be collected during May to August (Bhatia and Kungvankij 1971).

Based on studies of spawning activity under tank conditions, mature male and female fish separate from the school and cease feeding about a week prior to spawning. As the female attains full maturity, there ia an increase in play activity with the male. The ripe male and female then swim together more frequently near the water surface as spawning time approaches. The fish spawns repeatedly in batches for 7 days. Spawning occurs during late evening (1800–2200 hours).

Culture of Seabass

Seabass has been commercially cultivated in brackishwater and freshwater ponds and marine cages in many Southeast Asian countries. While the cagae culture technology is now established, grow-out techniques in pond are still are still in the developmental stages. Although considerable progress has been made over the past ten years, many problems remained unsolved.

The major problems that are always encountered during culture period are: (a) cannibalism during young stage (1–20 g), (b) dependence on trash fish as a main diet which has a very limited supply in many countries.

Despite some imperfections, the basic techniques of seabass culture are now developed and have been considered economically viable.

1. Culture techniques

As mentioned above, cannibalism is one of the most serious problems in seabass culture. High mortality is often encountered when uneven sizes of the fish are stocked. This has been noted to occur mostly where the fish are very young (1–20 cm in length, the first two months of culture). To minimize this problem, culture of seabass should be approached in two phases i.e. the nursery phase and the grow-out phase.

1.1 Nursery

The main purpose of the nursery is to culture the fry from hatchery (1–2.5 cm in size) to juvenile size (8–10 cm). This can solve the problem of space competition in the nursery tanks. Beyond the nursing period, the juveniles can be graded into different size groups and stocked in separate

grow-out ponds. It has been observed that the juveniles from the nurseries perform better in terms of growth and survival than those stocked directly into the grow-out ponds.

Nursing the fry in concrete tanks is not recommended as accumulation of excess feed on the bottom of the tank cannot be avoided. Such accumulation can cause bacterial disease. In addition, constant contact with the tank wall results in wounded fish and subsequent bacterial infection

1.1.1 Nursery pond design

Nursery pond size ranges from 500 to 2000 m^2 with water depth of 50–80 cm. The pond has separate inlet and outlet gates to facilitate water exchange. Pond bottom should be flat and sloping towards the harvesting or drainage gate. Inlet and outlet gates are provided with a fine screen (1 mm mesh size) to prevent predators and competitors from entering and fry from escaping the pond.

Fry ranging from 1–2.5 cm are suitable for stocking in the nursery ponds. Stocking density is between 20–50 individuals per square meter.

1.1.2 Pond preparation

A wellprepared pond is important as predators and competitors can endanger the stocked fry.

Some farmers still practice very crude farming techniques of drying the pond bottom and immediately filling with water and stocking fry directly for nursing. Feeding is entirely dependent on supplementary feed such as chopped or grounded trash fish and is done twice daily in the morning (1800 hours) and afternoon (1700 hours). In this method, the survival rate and growth rate are low.

To enhance production, the following improved pond preparation techniques are done: The nursery pond must be drained and dried until the bottom soil cracks to release toxic gases, oxidize mineralized nutrients, eradicate some pests and predators. In cases where the pond cannot be completely drained, derris root (rotinone) may be applied at the rate of 20 kg/ha toeradicate unwanted species. Derris root is prepared by cutting them into small pieces, crushing and soaking in water overnight. Only the solution is applied to the pond. If derris root is not available, a mixture of 50 kg/ha of ammonium sulfate (21-0-0) with lime at a ratio of 1:50 will be sufficient to weed out unwanted species. The mixture is applied to the portions of pond with water. The use of any chemicals or inorganic pesticides is not recommended because the residual effect remains for many years and can reduce the pond production. If pond soil is acidic, the pond bottom should be neutralized with lime before letting the water in.

Production techniques of juvenile in nursery ponds have been improved recently at Satul Fishery Station, Thailand. The improved technique is based on the live food production in the pond supplemented with chopped or grounded trash fish. After neutralizing pond bottom by liming, organic fertilizer (chicken manure) is applied at the rate of 500 kg/ha. Then water depth is gradually increased for the propagation of natural food. Two to three weeks prior to stocking,

newly-hatched Artemia nauplii are inoculated into the pond (1 kg of dry cyst/ha). Artemia will utilize the natural food as feed for growth and will reach adult stage within 10–14 days. The fry are immediately stocked at the rate of 20–50 individual per square meter.

Another approach to the improved technique is to stock Artemia nauplii in the separate pond and grow them into adult. Adults could be harvested daily to feed the fry.

1.1.3 Nursery pond management

Although seabass can be cultured in either freshwater or saltwater, fry must be acclimatized to the salinity and temperature prevailing in the pond on stocking to prevent loss.

Acclimatization is done in the following manner: transfer the fry to a tank, then gradually add nursery pond water. This can be completed within one day or more depending on the salinity difference. If the temperature and salinity in transport bag does not differ by more than 5°C and 5 ppt with the pond water, acclimation can be done by floating the bag in the pond for sometime to even out temperature difference. Pond water is then added gradually until both salinity become equal and the fry can be released.

Seabass fry are stocked in the nursery pond at a density of 20–50 fry/m^2. Stocking is usually done in the erly morning (0600–0900 hours) or early evening (2000–2200 hours) when the temperature is cooler.

Water replenishment is needed to prevent deterioration of pond water quality due to the decomposition of uneaten feed or excess growth of natural food. Normally, 30% of pond water is changed daily.

Supplementary feed is given daily. The feed used for nursing seabass is chopped and grounded (4–6 mm^3) trash fish, normally at the rate of 100% of biomass given twice daily in the first week (at 0900–1700 hours), gradually reduced to 60% for the second week and 40% in the third week. This has been found to be most effective feeding strategy for ponds without artemia inoculation.

The application of supplementary feed is a vital operational activity that should be done properly, if not, contamination of culture water and wastage of feeds result. Although the seabass in nature prefer live food, the fish can be trained to feed on dead animal. Prior to feeding, the fish should be attracted by sound (such as tapping a bamboo pole in the water) to induce them to form a school. Feeding time and place should be fixed. After the fish have formed a school, small amounts of feed are introduced by spreading into the water within the school of fish fry. It must be remembered that seabass never eat the feed when it sinks to the pond bottom. Therefore, feeding should be slow. When the fish are filled to satiation, they disappear thus feeding should be stopped. The same procedure should be followed at every feeding time. The first few days after stocking, feeding should be 5 to 6 times a day to teach them to accept dead feed. Once the fish is accustomed to it which takes about 5–7 days, feeding frequency is reduced to twice daily. In nurseries where Artemia is the main diet, once the Artemia population has thinned down, chopped or grounded trash fish can be supplemented using above described practice.

The nursing period lasts about 30–45 days until fingerling stage (size 5–10 cm). At this stage, they are ready for transfer to grow-out ponds.

1.1.4 Nursing in net cages

Nursing of seabass fry (1–2.5 cm to 8–10 cm) in cages is an approach to the nursery phase. The method has been successful since conducive environmental conditions such as flow through water, necessary for good health and growth of fish are used. It is likewise easy to maintain and require very little capital investment.

1.1.5 Nursing cage design and investment

The most convenient cage design is a rectangular cage made of synthetic netting attached to wooden frames. It is either (a) kept afloat by styrofoam, plastic or metal drum, or (b) stationary by fastening to a bamboo or wooden pole at each corner. The size of cages vary from 3 cubic meter (3 × 1 × 1m) to 10 cubic meters (5 × 2 × 1m). The mesh size of the net used for nursery cages is 1.0 mm. The cages may be installed in the river, coastal area or in a pond. Suitable sites for net cages should be free from biofoulers since the mesh size of a nursery cage is very small. Cages are easily damaged in strong currents and clogging by biofoulers. **(Fig. 20)**

1.1.6 Nursery cage management

Seabass fry (1–2.5 cm in size) are stocked in the nursery cage at the rate of 80–100 per square meter.

Stocking and feeding activity are the same as in nursery pond culture practice.

The net cages should be checked daily to ensure that the cages are not damaged by animals such as crabs or clogged with fouling organisms. Cleaning of the cages should be done every other day by brushing. This will allow water to pass through the cages naturally.

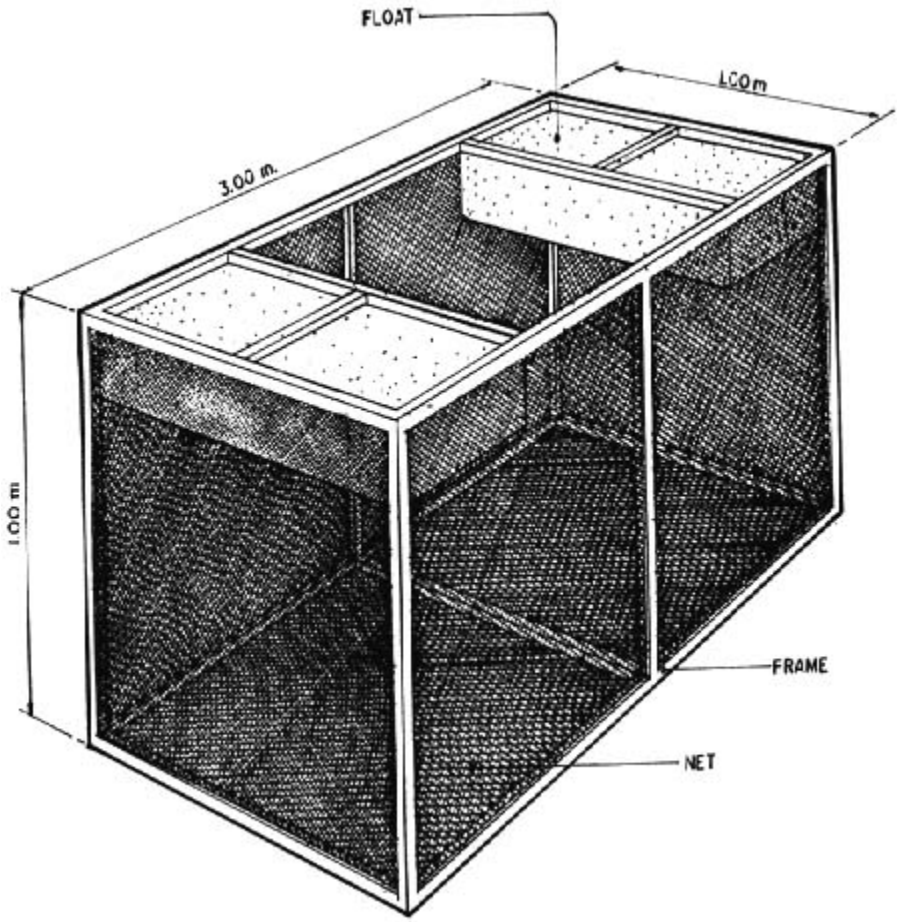


Fig. 20 Floating nursery cage for seabass.

After the nursing period of 30–45 days (in pond or cages) or when the fry have reached 5–10 gm, these are ready for transfer to grow-out ponds. Prior to stocking in grow-out ponds, grading procedure should be applied. Fish are graded into several sizes. It will give maximum advantage if the various sizes are stocked in separate ponds to prevent cannibalism.

1.2 Grow-out

The grow-out phase involves the rearing of the seabass from juvenile to marketable size. Marketable size requirement of seabass vary country to country e.g. Malaysia, Thailand, Hong Kong and Singapore. The normally accepted marketable size of seabass among these countries and region is between 700–1200 g while in the Philippines, marketable size is between 300–400 g. The culture period in grow-out phase also vary from 3–4 months (to produce 300–400) to 8–12 months.

2. Cage culture

Cage culture of seabass is quite well developed in Thailand, Malaysia, Indonesia, Hong Kong and Singapore. The success of marine cage culture of seabass and its economical viability have contributed significantly to large scale development of this aquaculture system

2.1 Suitable site for cage culture

Criteria for selecting a suitable site for cage culture of seabass include:

a. Protection from strong wind and waves. The cage culture site should preferably be located in protected bays, lagoons, sheltered coves or inland sea.
b. Water circulation. The site should preferably be located in an area where influenc of tidal fluctuation is not pronounced. Avoid installing cages where the current velocity is strong.
c. Salinity. Suitable site for seabass culture should have a salinity ranging from 13–30 ppt.
d. Biofouling. The site should be far from the area where biofoulers abound.
e. Water quality. The site should be far from the sources of domestic, industrial and agricultural pollution and other environmental hazards.

2.2 Design and construction of net cages

In general, square and rectangular cages with size varying from 20 to 100 m^3 are preferable because they are easy to construct, manage and maintain. Seabass cages usually are made of polyethelene netting with the mesh size ranging from 2 to 8 cm. The choice of mesh size depends on the size of the fish **(Table 8).**

There are two types of cages used in seabass culture:

(a) Floating cages

The net cages are attached to wooden, GI pipe or bamboo frames. The cage is kept afloat by floating material such as metal, plastic, styrofoam drum or bamboo. The shape of the cage is maintained with the use of concrete weights attached to the corners of the cage bottom **(Fig. 22).**The most manageable size for a floating cage is 50 m^3 (5 × 5 × 2m). This cage dimension is easy to change when clogged with fouling organisms.

(b) Stationary cages

The cage is fastened to the bamboo or wooden poles installed at its four corners **(Fig. 21).** Stationary cages are popularly used in shallow bays since they are easy to install.

2.3 Cage culture management and techniques

Prior to stocking seabass juvenile in cages, fish should be acclimatized to the ambient temperature and salinity prevailing in the cages. The fish should be graded into several size

groups and stocked in separate cages. The stocking time should be done in the early mornings (0600–0800 hours) or late in the evening (2000–2200 hours) when the temperature is cooler.

Stocking density in cages is usually between 40–50 fish per cubic meter. Two to three months thereafter, when the fish have attained a weight between 150–200 g, the stocking density should be reduced to 10–20 fish per cubic meter. **Table 9** shows the growth of seabass under varying densities in cages. There should be spare cages as these are necessary for transfer of stock and to effect immediate change of net in the previously stocked cage once it has become clogged with fouling organisms. Changing cages allows for grading and controlling stock density.

2.4 Feeds and feeding

Feed is the major constraint confronting the seabass culture industry. At present, trash fish is the only known feed stuff used in seabass culture. Chopped trash fish are given twice daily in the morning at 0800 hours and afternoon at 1700 hours at the overall rate of 10% of total biomass in the first two months of culture. After two months, feeding is reduced to once daily and given in the afternoon at the rate of 5% of the total biomass. Food should be given only when the fish swim near the surface to eat.

Table shows The choice of netting mesh size of fish.

mesh size	size of fish
0.5 cm	1–2 cm
1 cm	5–10 cm
2 cm	20–30 cm
4 cm	bigger than 25 cm

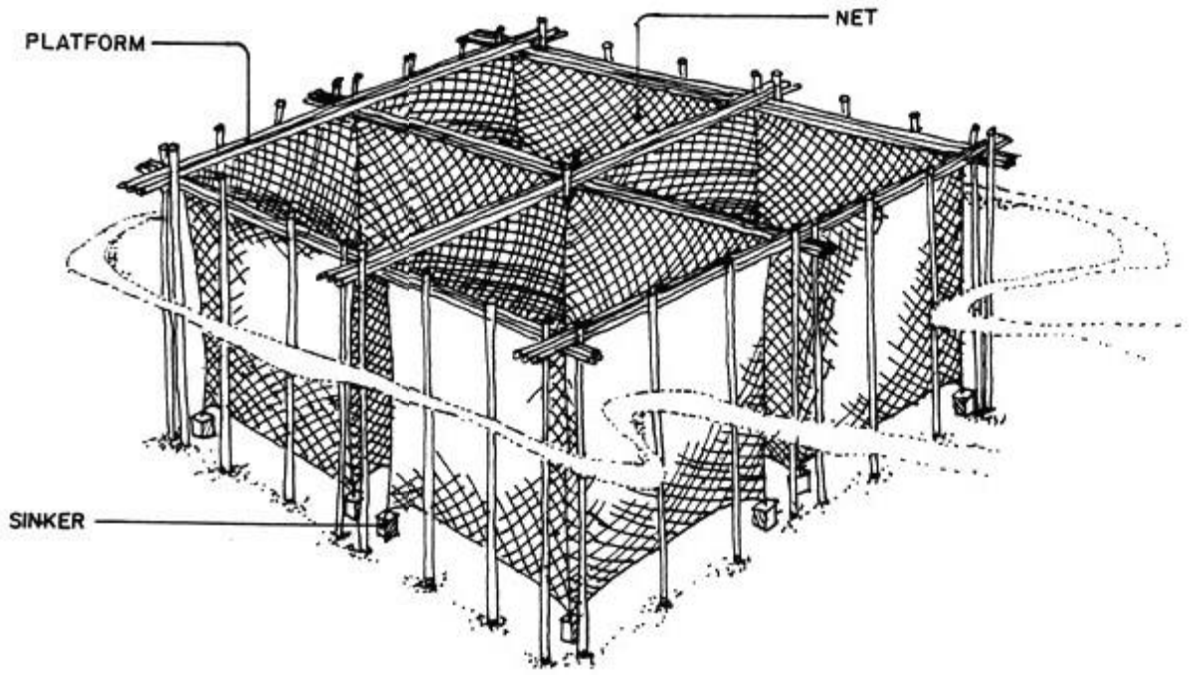
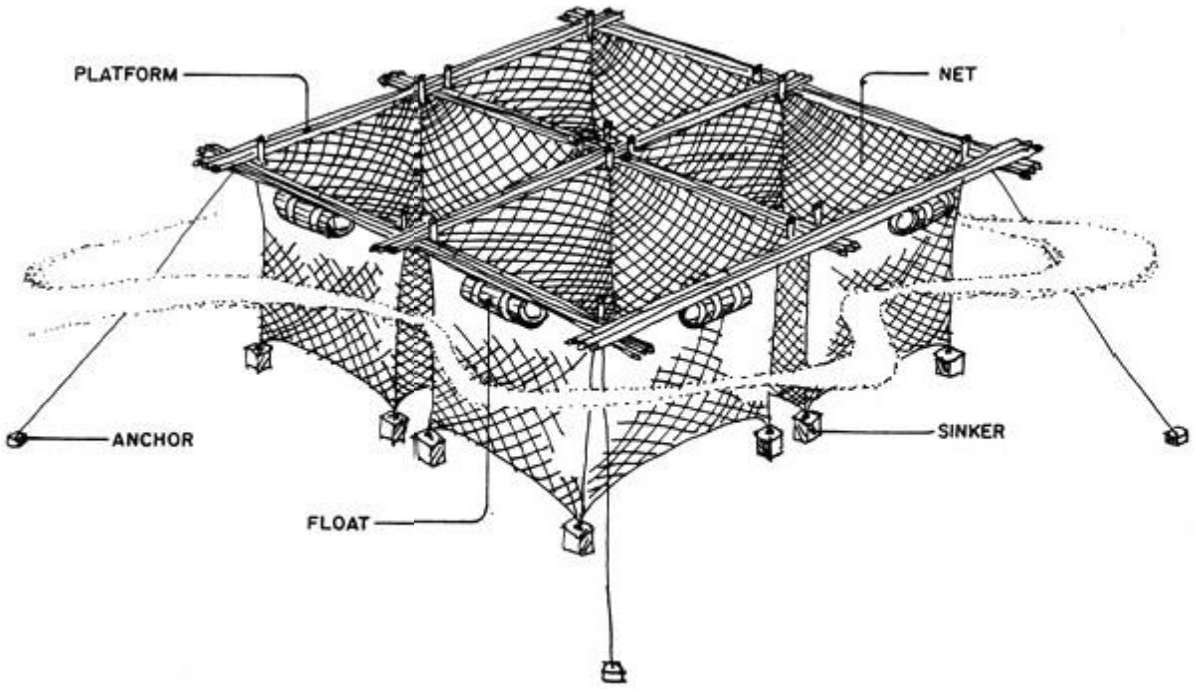


FIG. 21 STATIONARY CAGES



FLOATING CAGES

Table shows the Monthly growth of seabass at different stocking densities in cages.

Culture period (month)	Stocking density		
	16/m^2	24/m^2	32/m^2
0	67.80 g	67.80 g	67.80 g
1	132.33 g	137.53 g	139.20 g
2	225.20 g	229.10 g	225.50 g
3	262.88 g	267.50 g	264.11 g
4	326.15 g	331.97 g	311.50 g
5	381.08 g	384.87 g	358.77 g
6	498.55 g	487.06 g	455.40 g

Since the supply of trash fish is insufficient and expensive in some countries, its use is minimized by mixing rice bran or broken rice to the trash fish **(Table 10).** However, even with these cost cutting measures, feed cost remain quite high.

A very recent development on improving the dietary intake of seabass is the introduction of moist feed. So far, the use is still on experimental stage. The feed composition recommended is presented at **Table 11.**

2.5 Fish cage management

Regular observation of cages is required. Since fish cages are immersed under water all the time, they are vulnerable to destruction by aquatic animals such as crabs, otter, etc. If damaged, they should be repaired immediately or replaced with a new one.

In addition to biofouling, the net walls of cages are subjected to siltation and clogging. Biofouling is unavoidable since the net walls usually represent a convenient surface for attachment by organisms such as amphipod, polycheate, barnacles, molluscan spats, etc. These could lead to clogging and reduce exchange of water and may result in unnecessary stress to the cultured fish due to low oxygen and accumulation of wastes. Feeding and growth would likewise be affected.

To date, mechanical cleaning of fouled nets is still the most efficient and cheap method. In areas where fouling organisms are abundant, rotational usage of net cage is highly recommended.

3. Pond culture

Although methods of pond culture of seabass have been practiced for over 20 years in Southeast Asia and Australia, not much has been done on the commercial scale. At present, culture of seabass in brackishwater pond has been identified in some countries as having tremendous market potential and high profitability. These, however, can be achieved if conditions are met such as adequate fry supply, availability of suitable site and properly designed fish farm. Supply of fry from the wild is very limited. As with cage culture, it is one of the constraints in the intensification of seabass culture in ponds. However, with the success in artificial propagation of seabass, fry supply may largely come from this source in the future. A comparison of hatchery bred and wild fry cultured in ponds did not show very significant difference in growth rate**(Table 12).**

There are two culture systems employed in pond culture of seabass:

(a) Monoculture

Monoculture is that type of culture where a single species of animal is produced, e.g. seabass. This culture system has a disadvantage. It is entirely dependent on supplementary feeding. The use of supplementary feed reduces profit to the minimal, especially where the supply of fresh fish is limited and high priced.

Table shows the Combination of feed stuff.

Ingredient	Percentage
Trash fish	70%
Rice bran or broken rice	30%

Table shows the Combination of moist diet

Ingredient	Percentage
Fish meal	35%
Rice bran	20%
Soy bean meal	15%

Corn meal	10%
Leaf meal	3%
Squid Oil (or fish oil)	7%
Starch	8%
Vitamin mix	2%

Table shows Comparison of growth rate of seabass (Lates calcarifer) culture in pond between wild fry and hatchery bred fry at stocking density of 3/m^2.

	Wild		Hatchery bred	
	B.L.	B.W.	B.L.	B.W.
Stocking	10.5 cm	40.44 g	5.2 cm	5 g
1st month	13.0	88.9	7.6	12.0
2nd month	16.4	204.2	10.6	26.02
3rd month	20.9	276.3	15.2	118.1
4th month	23.4	326.5	19.5	220.9
5th month	24.1	385.2	21.8	280.6
6th month	28.2	453.5	23.2	349.6

(b) Polyculture

This type of culture approach shows great promise in reducing if not totally eliminating the farmers' dependence on trash fish as food source. The method is achieved by simply incorporating a species of forage fish with the main species in the pond. The choince of forage fish will depend on its ability to reproduce continuously in quantity sufficient to sustain the growth of seabass throughout the culture period. The forage fish must be such a species that could make use of natural food produced in the pond and does not compete with the main species in terms of feeding habit such as Oreochromis mossambicus, Oreochromis niloticus, etc.

4. Criteria in the selection of site for seabass culture

4.1 Water supply

The site should have enough good water quality supply all year round. Water quality includes all physico-chemical and microbiological characteristics of water being used for culture of seabass. The following are the parameters normally considered as suitable water supply:

Parameter	Range
pH	7.5–8.5
Dissolved oxygen	4–9 ppm
Salinity	10–30 ppt
Temperature	26–32°C
NH_3	less than 1 ppm
H_2S	less than 0.3 ppm
Turbidity	less than 10 ppm

4.2 Tidal fluctuation

Area best suited for seabass should have moderate tide fluctuation range between 2–3 meters. With this tidal characteristic even for ponds as deep as 1.5 meters, complete drainage during low tide can be done. In addition, the pond can readily admit water during spring tide.

4.3 Topography

It is advantageous if the selected site is mapped topographically. This would reduce development and operational costs such as for water pumping.

4.4 Soil

Ideally, the soil at the proposed site should have enough clay content to ensure that the pond can hold water. Area with acid sulphate soil should be avoided.

4.5 Accessibility

Accessibility is an important consideration in site selection for logical reasons. Overhead cost and delay in the transport of material and product may be minimized with good site accessibility.

Other factors in the selection of site that should be considered include availability of seed, labour, technical assistance, market demand and suitable social condition.

5. Pond design and construction

Seabass ponds are generally rectangular in shape with size ranging from 2000 m^2 to 2 hectares and depth of 1.2 to 1.5 meters. Each pond has separate inlet and outlet gate to facilitate water exchange. The pond bottom is entirely flat levelling toward the drainage gate **(Fig. 23).**

6. Pond preparation

Preparation of grow-out ponds is similar to the procedure followed in pond system. In monoculture, the fish are stocked immediately after neutralizing the pond soil with lime. Ponds are filled immediately after pond preparation.

In polyculture, after the pond soil is neutralized, organic fertilizer (chicken manure) is applied at the rate of 1 ton per hectare. Then water depth is gradually increased for propagation of natural food. When abundance of natural food are observed, selected tilapia broodstocks are released to the pond at the rate of 5,000–10,000 per hectare. Sex ratio of male to female is 1:3. The tilapia are reared in pond for 1 to 2 months or until tilapia fry appear in sufficient number. Seabass juveniles are then stocked.

Seabass juveniles (8–10 cm in size) from nursery are stocked in the grow-out pond at the rate of 10,000–20,000 per hectare in monoculture and 3,000–5,000 per hectare in polyculture system. Prior to stocking, juveniles are acclimatized to pond culture and salinity conditions. Stocking the fish in uniform sizes will be most ideal and should be done at cooler times of the day.

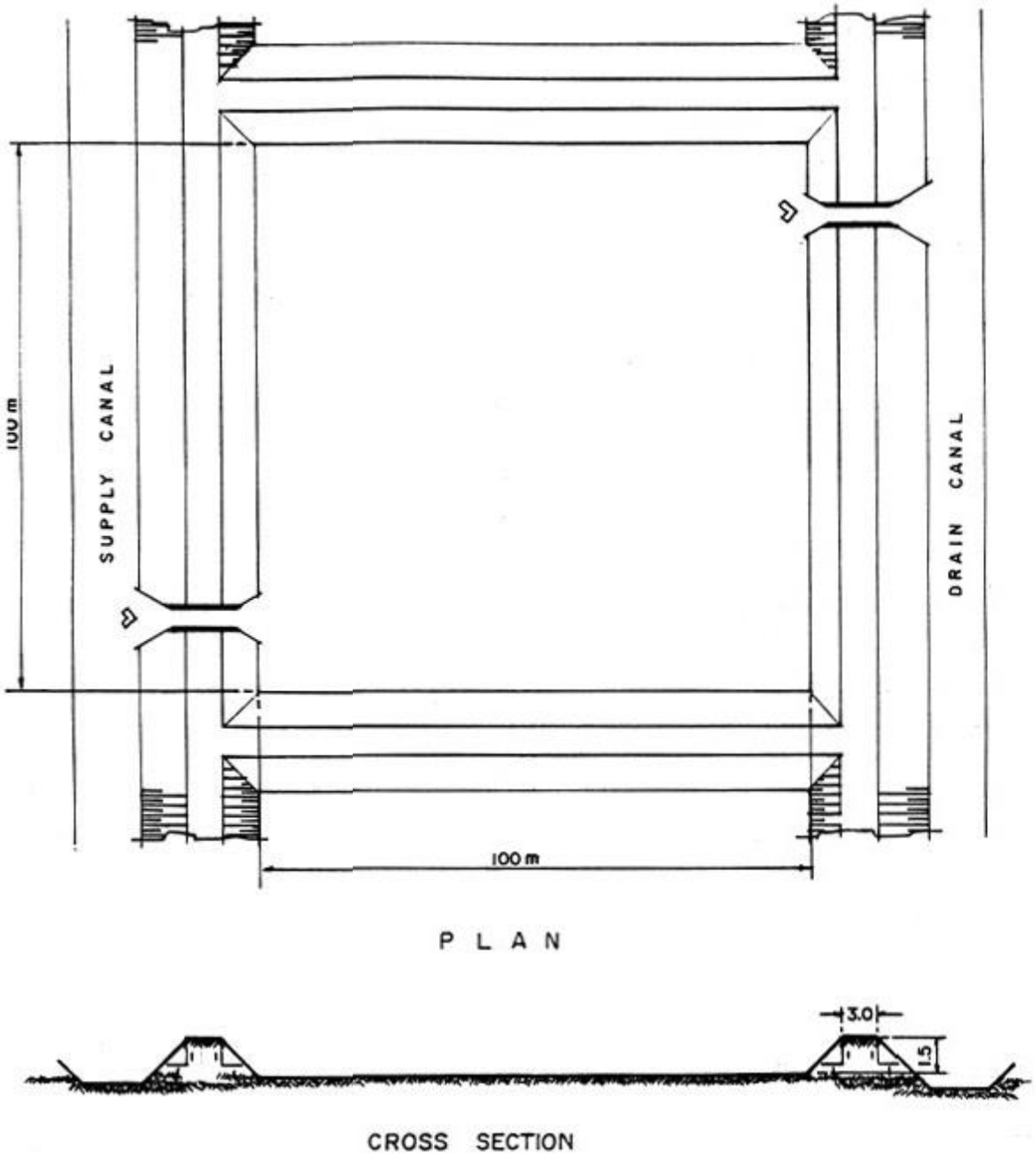


Fig. 23 Pond lay-out for seabass culture.

7. Pond management

Due to the need of maintaining r atural food in ponds, water replenishment in p lyculture system should be minimized. Water change should be done once in three days f cr about 50% of capacity.

However, in monoculture where supplemental feed is given daily, there are chances that excess feed may pollute the water. Hence, daily water replenishment is necessary.

8. Feeds and feeding

Supplementary feed is not required in the polyculture system, but in monoculture, daily feeding is a normal practice. The method of supplying feed in ponds follows often the practice employed in cage culture.

Conclusion

Seabass (Lates calcarifer) culture enterprise is one of the most dynamic and potentially profitable segments of the brackish and marine water fish farming industry in Southeast Asia. It is a desirable fish with good flesh texture and taste, high market value and market value and demand. It can be reared both in freshwater and seawater conditions. In the past 5 years, over 10,000 farmers engaged in cage culture of seabass and over 20,000 hectares of land have been established in the Region for intensive pond production of the species.

PRAWN/SHRIMP CULTURE

INTRODUCTION

Coastal aquaculture has been identified by the Government of India as high potential area for increasing the fish and shell fish production and also to achieve economic and social benefits . India with over 8,100 Km of coastline, vast stretches of estuaries/ backwaters, lagoons provide enormous opportunities for brackish water shrimp farming.

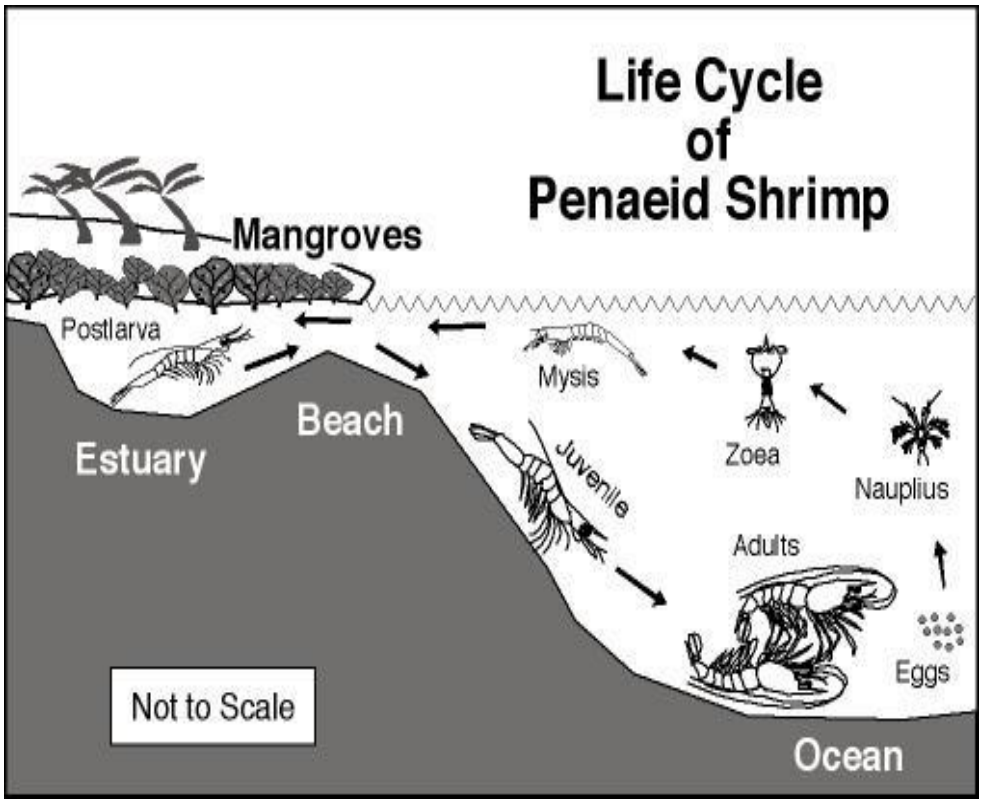
Commercial shrimp farming is almost three decades old in India. During the early nineties due to proven technology in post larvae production and farming of two varieties of shrimps viz white shrimp (*Penaeus indicus*) and tiger shrimp (*Penaeus monodon*).Large scale growth of shrimp farms and hatcheries was witnessed during a short span of ten years. It is time for Blue Revolution to exploit the huge potential in fisheries sector.

Shrimps are called the "Pinkish Gold" of the sea because of its universal appeal, unique taste, high unit value realisation and increasing demand in the world market. Scope for brackish water shrimp farming. The over exploitation of shrimp from coastal waters and the ever increasing demand for shrimp and shrimp products in the world market has resulted in the wide

gap between the demand and supply in the International market. This has necessitated the need for exploring newer avenues for increasing shrimp production. Water quality parameters required for maximum feed efficiency and maximum growth of *Penaeus monodon* are given below :

Water Parameters	Optimum level
Dissolved Oxygen	3.5-4 ppm
Salinity	10-25 ppt
Water Temperature	26-32 degree centigrade
Ph	6.8-8.7
Total nitrite nitrogen	1.0 ppm
Total ammonia (less than)	1.0 ppm
Biological Oxygen Demand (BOD)	10 ppm
Chemical Oxygen Demand (COD)	70 ppm
Transparency	35 cm
Carbon dioxide (less than)	10 ppm
Sulphide (less than)	0.003 ppm

Penaeus monodon



S.NO		Traditional (within CRZ)	Extensive (outside CRZ)
i.	Farm Size	5 ha	5 ha
ii.	Culture period	5 - 6 months	5 - 6 months
iii.	Stocking density (PL-20)	60,000/ ha	1,00,000/ ha
iv	Survival	70%	70%
v	Expected production	1.0-1.5 tonnes/ha/crop	1.5-2.5 tonnes/ha/corp
vii	**Price of shrimp has been taken as Rs.600/kg**		

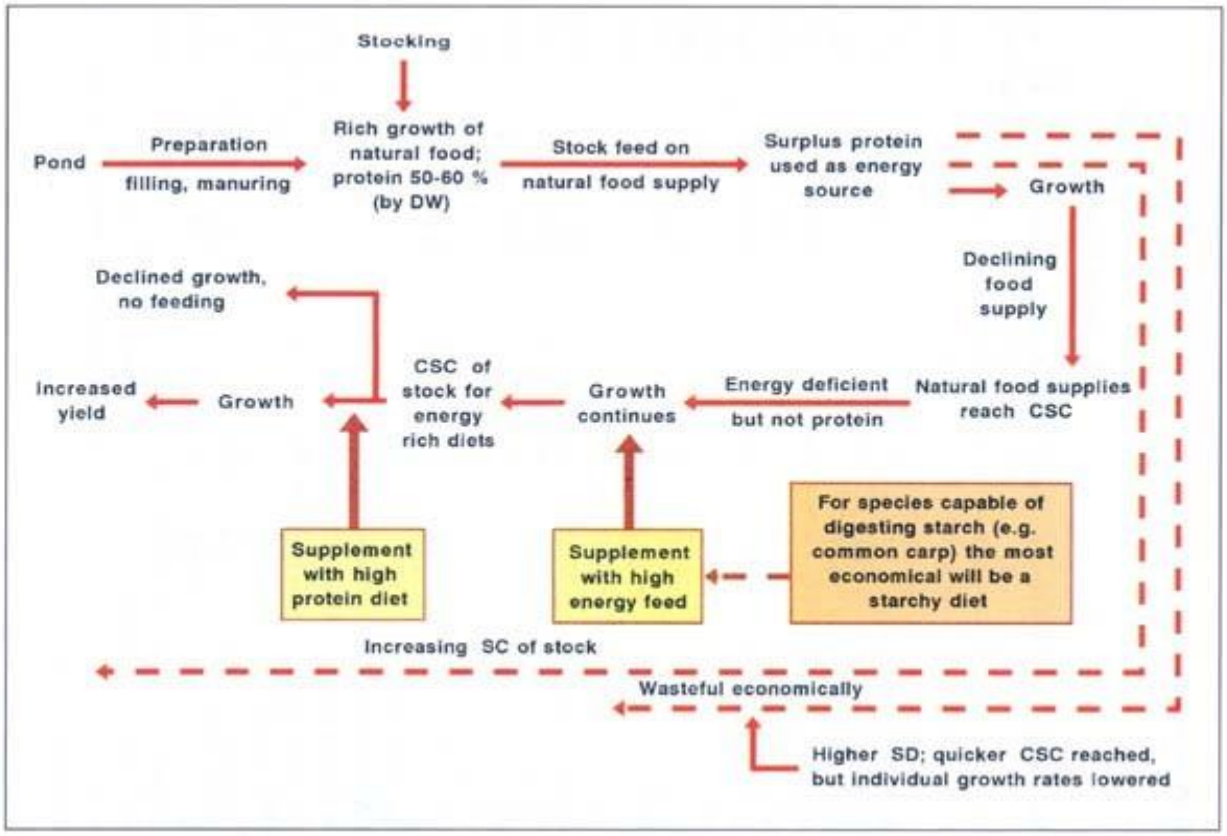
Traditional aqua farming

Technical Parameters for establishing a extensive shrimp farming

Design and Construction of shrimp farm :

- An extensive shrimp farm should be of the size 0.4 - 0.5 ha. and preferably drainable
- The ponds generally should have concrete dikes, elevated concrete supply canal with separate drain gates and adequate life supporting devices like generators and aerators.
- The design, elevation and orientation of the water canals must be related to the elevation of the area with particular reference to the mean range of tidal fluctuation.
- The layout of the canals and dikes may be fitted as closely as technically possible to existing land slopes and undulation for minimizing the cost of construction.

SEMI-INTENSIVE FARMING

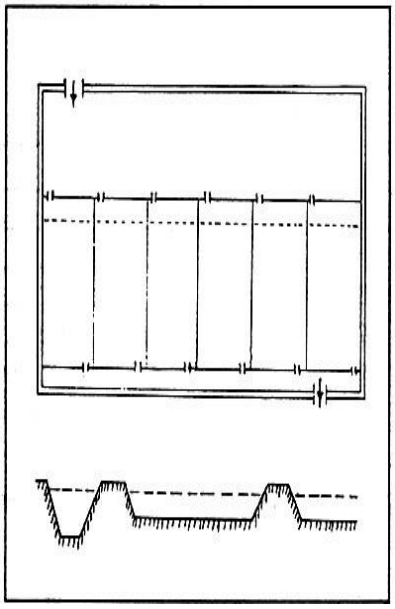


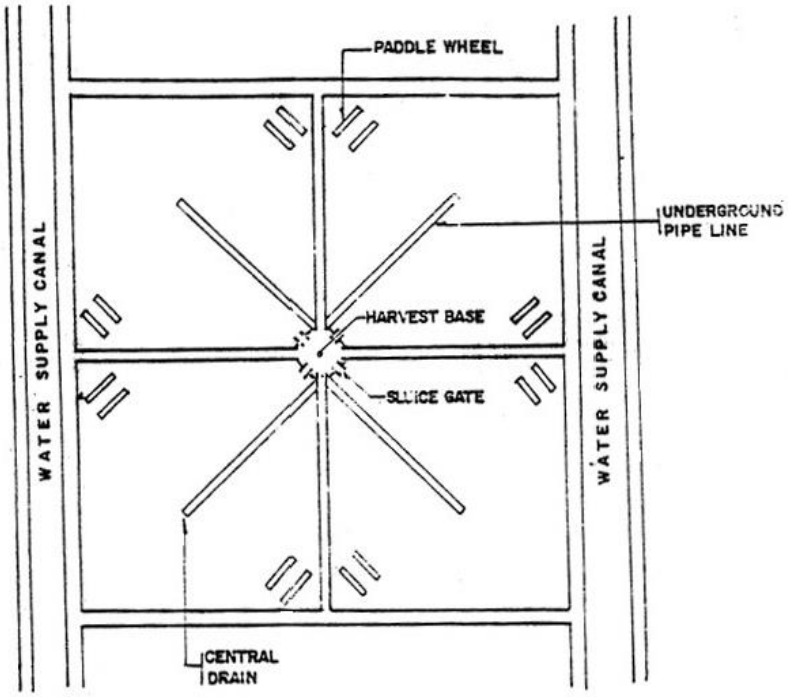
EARTH WORK

It is normally carried out in the following order

- Site clearing
- Top soil stripping
- Staking of centre lines and templates
- Preparation of dike foundation, excavation of drainage canals
- Construction of dikes (peripheral and secondary)
- Formation and compaction of dikes, Excavation of pits for gatesa
- Levelling of pond bottom
- Construction of gates and refilling of pits,
- Construction of dike protection

The top soil may be set aside and should again be spread later to preserv pond bottom fertility.





THE ESSENTIAL COMPONENTS OF A SHRIMP FARM

- Ponds
- Water intake structure
- Store room for feed and equipments
- An area for cleaning of the harvest
- Pump house
- Watch and ward room,
- Office and A mini laboratory.

POND PREPARATION

- Proper pond preparation will ensure higher productivity and production levels. The main objectives of pond preparation are :
- To eradicate weed fishes and other harmful organisms
- To remove abnoxious gases
- To improve the natural productivity of the pond eco system
- To maintain high water quality for proper growth and higher survival percentage.

- Eradication of unwanted organisms is usually carried out by draining out the entire water and drying the pond bottom till it cracks
- This also helps in removal of obnoxious gases and oxygenation of the pond bottom.
- It also improves the fertility of the soil.
- Liming is done for correcting the pH and to kill pathogenic bacteria and virus.
- In undrainable ponds mahual oil should be applied @ 200 ppm to eradicate the weed fishes.
- After around two weeks organic and inorganic fertilisers are applied to enrich the soil and water.
- Once the thick lab-lab is formed the water level is raised and the pond is made ready for stocking.

ACCLIMATION

SELECTIVE STOCKING

The most suitable species for culture in India are the Indian white shrimp *Penaeus indicus* and

Tiger shrimp *P. monodon*. The stocking density varies with the type of system adopted and the species selected for the culture. Shrimp farming with a production range of 1.5 to 2.5 tonnes/ha/crop with stocking density upto 1,00,000/ha/crop viz; 10 Nos./m^2 may be allowed. In

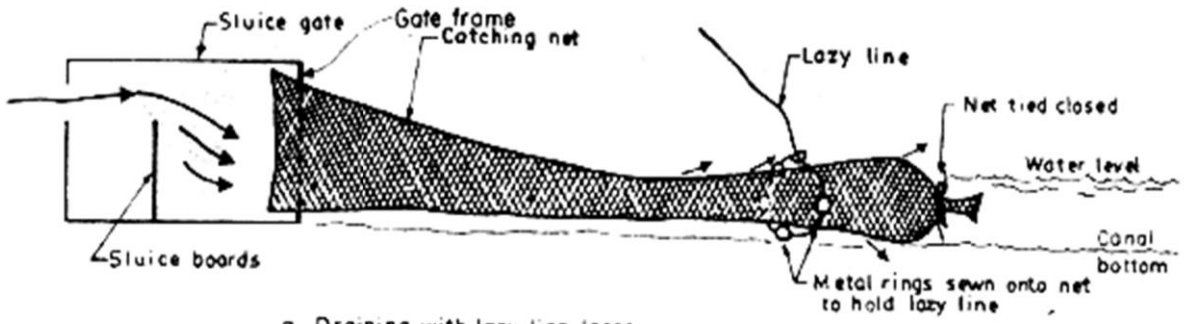
order to have uniform growth it is always advisable to go in for hatchery reared and PCR tested seeds.

Food and feeding Shrimp diets may be supplementary or complete. In a extensive system the shrimps need a complete diet. Although natural food items have good conversion values, it is difficult to procure in large quantities and maintain a continuous supply. At present most of the aquaculture farms depend on imported feed with a FCR of 1:1.5 - 1.8. The feeding could be done by using automatic feed dispensers, or by broadcasting all over the pond. If feeding trays are employed in selected pockets in the pond wastage of feed can be reduced.

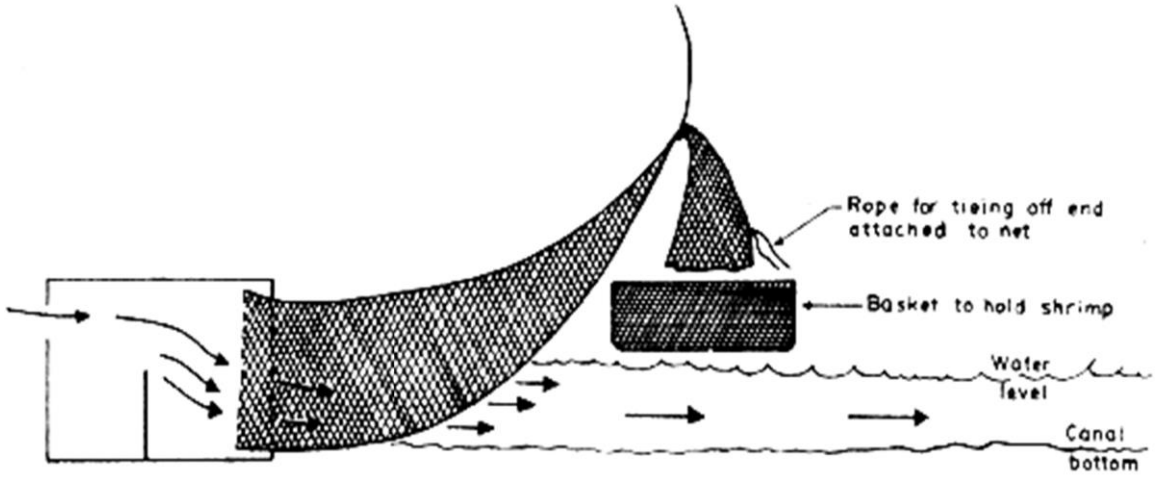
Ingredients:	Contents:
Wheat (61% starch)	17.7
Wheat flour (88%) starch	4.4
Wheat gluten:	5.6
Fishmeal (LT):	18.9
Soya (hi-pro)	46.9
Shrimp meal	4.9
Oil	1.6

HARVESTING

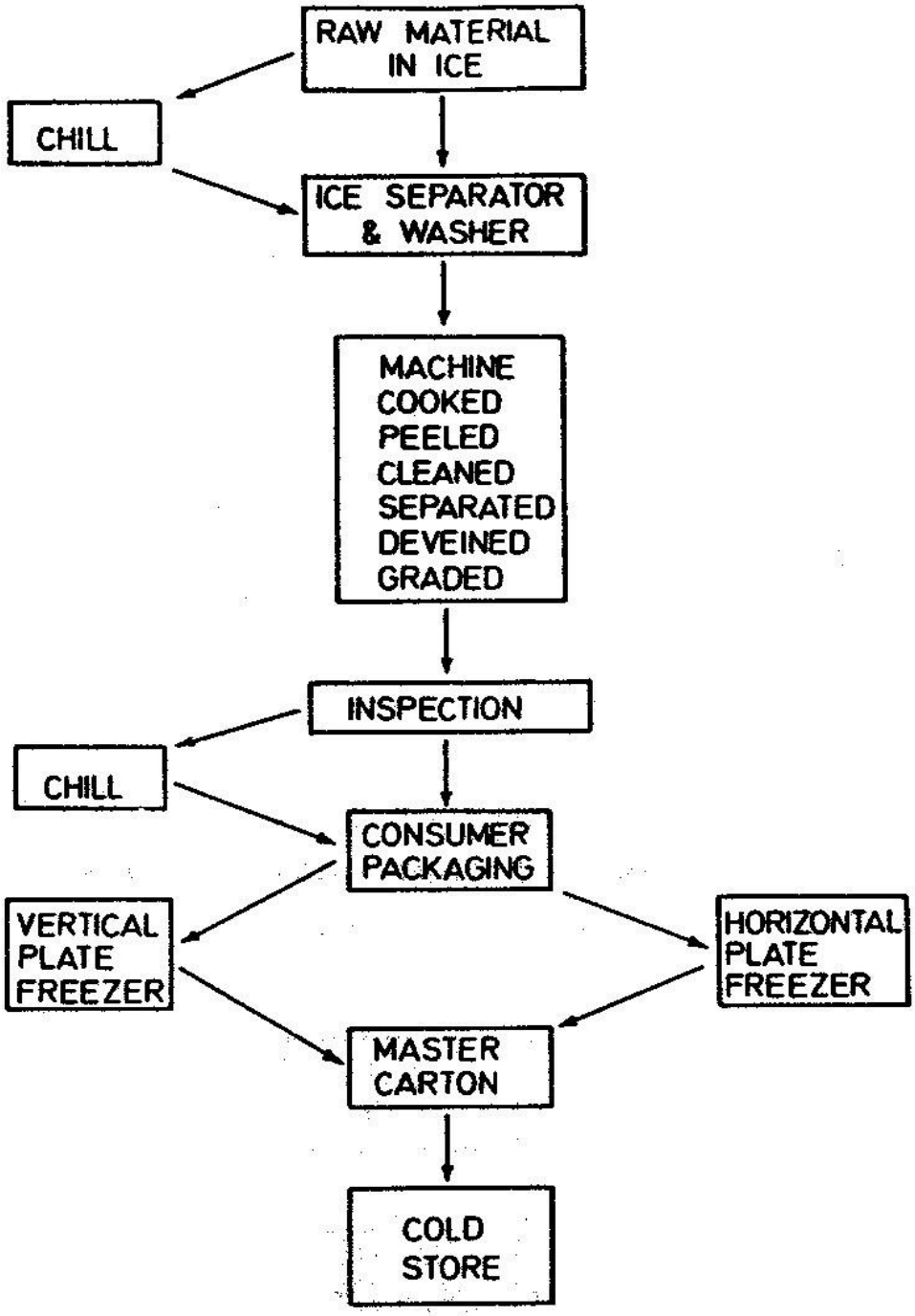
Complete harvesting can be carried out by draining the pond water through a bag net and hand picking. The average culture period required is around 120 days during which time the shrimps will grow to 25-35 gm size (depending on the species). It is possible to get two crops in a year. Harvested shrimps can be kept between layers of crushed ice before transporting the consignment to market.



a. Draining with lazy line loose



b. Draining with lazy line tightened and bag of net pulled to bank of drain canal to remove shrimp



MARKETING

Because of huge gap between supply and demand of shrimps in local as well as international market, there may not be any problem in marketing. Shrimp can either be sold directly by the

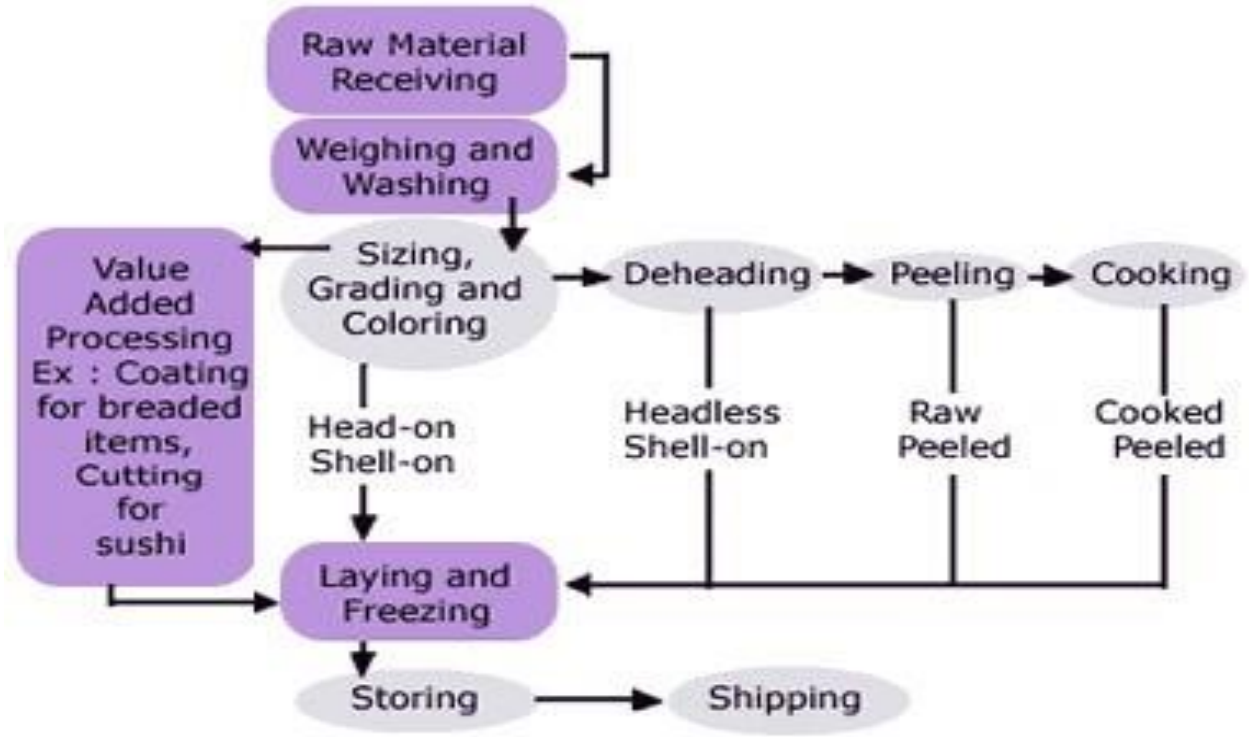
farmers in the market or sold to exporters for processing. Shrimp can be exported in frozen form with head on, head less, battered and breaded, or IQF products or any other form with value addition depending on the requirement of the buyer.

WORLD FISH MARKET AT A GLANCE

	2009	2010 *estim.*	2011 *f'cast*	Change: 2011 over 2010
	million tonnes			%
WORLD BALANCE				
Production	**144.8**	**146.9**	**151.7**	**3.2**
Capture fisheries	89.1	87.7	90.1	2.7
Aquaculture	55.7	59.2	61.6	4.0
Trade value (exports USD billion)	**95.7**	**107.5**	**119.7**	**11.3**
Trade volume (live weight)	**54.9**	**55.2**	**56.0**	**1.4**
Total utilization	**144.8**	**146.9**	**151.7**	**3.2**
Food	118.0	121.1	124.0	2.5
Feed	20.0	17.7	20.3	14.4
Other uses	6.8	8.1	7.3	-9.3
SUPPLY AND DEMAND INDICATORS				
Per caput food consumption:				
Food fish (kg/year)	17.3	17.6	17.8	1.3
From capture fisheries (kg/year)	9.1	9.0	9.0	-0.2
From aquaculture (kg/year)	8.2	8.6	8.8	2.8

FAO FISH PRICE INDEX[1] (2002-2004=100)	2009	2010	2011 *Jan-Oct*	Change: Jan-Oct 2011 over Jan-Oct 2010 %
	126	137	152	16.4

[1] Data source: Norwegian Seafood Export Council


Raw Material Receiving
Weighing and Washing
Sizing, Grading and Coloring
Value Added Processing Ex : Coating for breaded items, Cutting for sushi
Deheading
Peeling
Cooking
Head-on Shell-on
Headless Shell-on
Raw Peeled
Cooked Peeled
Laying and Freezing
Storing
Shipping

==========

FRESHWATER PRAWN FARMING

1. Introduction

Indian aquaculture has been evolving from the level of susbsistence activity to that of an industry. This transformation has been made possible with the development and standardization of many new production and associated techniques of input and output subsystems. In recent years aquaculture has created great enthusiasm and interest among entrepreneurs especially for shrimp farming in coastal areas. Shrimp farming is capital intensive activity and uncontrolled mushrooming growth of it has led to outbreak of diseases and attributed environmental issues calling for closure of shrimp farms.

Although India has vast freshwater resources they are not fully exploited except for carp culture in limited scale. Fresh water fish culture employing composite fish culture technology has become popular for use in large number of tanks and ponds in the country. To meet the raw material required by the processing units for export demand there is urgent need to expand our production base. In addition it is always stressed that there is a need to utilise our natural resources productively to ensure the much needed food security.

2. Scope for Fresh Water Prawn Culture

Considering the high export potential, the giant fresh water prawn, Macrobrachium rosenbergii, the scampi, enjoys immense potential for culture in India. About 4 million ha. of impounded freshwater bodies in the various states of India, offer great potential for fresh water prawn culture. Scampi can be cultivated for export through monoculture in existing as well as new ponds or with compatible freshwater fishes in existing ponds. It is exported to EEC countries and USA. Since the world market for scampi is expanding with attractive prices, there is great scope for scampi production and export.

3. Technical Parameters

The giant freshwater prawn is suitable for cultivation in tropical and subtropical climates. It is a hardy species by virtue of its ability to adapt to various types of fresh and brackish-water conditions. It accepts pelleted feed and has omnivorous feeding habit. In the natural enviroment, lower reaches of rivers, tidal inlets, where water is directly or indirectly connected with sea are their preferred habitat specially during spawning. The breeding takes place in low saline waters which is also needed for larval and post larval development after incubation. Breeding of M.rosenbergii takes place in estuaries.

Though seed may be available in natural sources to a limited extent, for large scale culture there is a need to ensure regular supply of seed. For ensuring availability of quality seed in predictable quantity freshwater prawn hatcheries should be encouraged, technology for which is already developed. Freshwater prawn hatcheries are coming up in many states.

The techno-economic parameters required for establishment of prawn farm and its successful operation are briefly described in this booklet. The parameters are averaged out and the costs are only illustrative.

3.1. Site selection

The site selection plays an important role as the entire management aspect of the farm ultimately depends on specific conditions of the site. The aspects to be considered are topography of the area, soil type, availability of quality water etc.. The area should be free from pollution and flooding. Other considerations like approach roads etc. have also to be taken into account.

3.2 Soil quality

The ideal soil for Macrobrachium culture should be clay silt mixture or sandy loam comprising of 60% sand and 40% silt with good water retention capacity.

3.3. Water quality

There should be availability of abundant and good quality water.The water should be free from any kind of pollution. The pH should be maintained at 7 to 8.5. The temperature should range from 18^0 to 34^0C with an optimum range of 27^0 C to 31^0 C. Dissolved oxygen content should be higher than 75% saturation.

3.4 Pond construction

Rectangular ponds are suitable mainly from the harvesting point of view. A convenient width is 30-50 m, whereas length of the pond depends on site, topography and farm layout. Normally a size of 0.5 to 1.5 ha is found suitable. The average depth of the ponds should be 0.9m with a minimum of 0.75m and a maximum of 1.2m. Dike and pond slope may be kept at 2:1. Bund must have a freeboard of at least 60 cm above the highest water level in the pond. Designing and layout of the farms may be done keeping in view the water intake

and water outlet facilities. The drainage system should be designed carefully to prevent mixing of outlet water with incoming water.

3.5. Water supply and drainage

Appropriate water supply and drainage systems have to be designed keeping in view the water source and topography of the area. Tubewell and pumping system may be considered if required for water intake/exchange. Water exchange on weekly or fortnightly basis as required is desirable and provisions are to be made accordingly.

4. Farm Management

The type of pond preparation to be adopted before stocking is based on the type of culture and its intensity and nature of the culture pond. Liming of the pond assumes great importance here than in the case of freshwater fish culture. The application of fertilisers is restricted in case pelletised feed is used. However, occasionally cow dung, single super phosphate, urea etc. can be applied on assessing the productivity.

The stocking density normally varies from 4000 to 50000 nos. of post larvae per ha depending on the type and intensity of the management practices. The culture system may be monoculture or polyculture with carps. In case of polyculture with carps the more pond depth is preferred at 4-5 feet. In case of polyculture the stocking density of prawn may vary from 2500-20000 post larvae. The carp fingerlings may be of the order of 5000 - 2500 Nos. Nursery may be incorporated where the post larvae obtained from hatcheries could be reared for a period of 4-5 weeks till they attain 40-50 mm or 1-3 gram.

In order to get desired production, feeding, aeration, water exchange, periodic monitoring should be continued. The quality and type of feed is based on culture system. Macrobrachium with its omnivorous feeding habits can make use of a variety of feeds from common wet feed made from rice bran and oil cake to scientifically formulated pelleted feed. The rate of feeding is determined by the stage of growth of prawn, water quality, density of stock and other manuring practices. Generally the feeding rate my be 5% of the body weight.

The duration of culture varies from 6 to 12 months depending on the type of culture practice. Generally in monoculture the culture period may be 6-8 months under monoculture and 8-12 months under polyculture. The average growth of prawn may range from 50 gms to 200 gms depending on the duration, density, water quality, feeding etc. The survival rate may range 50% to 70% depending on the type of management practices.

5. Extension services

The borrower should have experience in prawn farming and should be conversant with production technology, trade etc. Fish Farmers Development Agencies (FFDA) have been established in almost all districts for providing necessary training. The offices of Marine

Products Export Development Authority (MPEDA) in most of the coastal states also provide necessary assistance.

6. Marketing

There is good demand for fresh water prawn in both local and international markets, as such there may not be any problem in marketing the same. Fresh water prawns can be sold directly by the farmers either in the market or to exporters for processing before export.

7. Financial outlay

Details for the financial outlay have been indicated in Annexure I. It can be seen therefrom that the capital cost for a 1 ha. unit has been estimated as Rs. 2.075 lakh while the operational cost for one crop works out to Rs.1.214 lakh. The items and cost indicated under the model are indicative and not exhaustive. While preparing projects for financial assistance the costs have to be assessed taking into account actual field conditions.

8. Margin money and bank loan

The entrepreneur is expected to bring margin money out of his own resources. The rates of margin money stipulated are 5% for smaller farmer, 10% for medium farmer and 15% for other farmers. For corporate borrowers the margin stipulated is 25%. NABARD could consider providing margin money loan assistance in deserving cases.

9. Rate of Refinance

NABARD provides refinance assistance for freshwater prawn farming to commercial banks, cooperative banks and Regional Rural Banks. The rate of refinance is fixed by NABARD from time to time.

10. Financial viability

The following assumptions have been made for working out the financial viability of the project.

i)	Farm size	1 ha.
ii)	Culture period	6-8 months
iii)	Stocking density	30,000 /ha
iv)	Survival	60%
v)	Feed conversion ratio	2.5:1
vi)	Expected production	1260 kg/ha/crop
vii)	Only one crop of 6-8 months culture period has been considered	
	Sale price of prawn has been taken as Rs. 170 per kg. The financial analysis has been shown in Annexure I.	
	The results of analysis are:	

	i) NPW at 15% Discounting = Rs. 2.33 lakhs	
	ii) BCR at 15% Discounting = 1.37:1	
	iii) IRR = 77%	

11. Rate of interest

Interest rate to be charged would be as indicated by bank/RBI/NABARD from time to time.

12. Repayment period

The borrower will be able to repay the bank loan in 8 years (Annex -I) with a grace period of one year on repayment of the principal.

13. Security

Security from the ultimate beneficiaries may be obtained as per the guidelines of RBI issued from time to time.

Annexure - I

ESTIMATED FINANCIAL OUTLAY FOR GIANT FRESH WATER

PRAWN (MACROBRAHIUM RESENBERGII) CULTURE IN 1 HA WATER AREA

A. Capital Cost

		Units	Quantum	Rate (Rs.)	Total
1	Construction of pond including digging, bund construction and compaction and consolidtion	Cum	7500	15	112500
2	Shallow tubewell and pumpset 5 HP	Nos		L/s	35,000
3	Pump house cum store room-AC roof			L/s	20,000
4	Inlet/outlet sluices				10,000
5	Nets and other implements			L/s	10,000
6	Aerator	Nos	1	15,000	15,000
7	Miscellaneous including laying of pipe line etc.			L/s	5,000
	Total A				**207,500**

B. Operational cost for one crop (6-8 months)

		Units	Quantum	Rate (Rs.)	Total

1	Lime	Kg	300	5	1,500
2	In organic fertiliser (super phosphate)	kg	75	5	375
3	Fertiliser - Organic-Cow Dung	tons	2	300	600
4	Seed	Nos	30,000	0.6	18,000
5	Feed-pelletted feed	Kgs	3,150	20	63,000
6	Pumping and aeration charges			L/s	10,000
7	Watch and Ward	Mandays	240	40	9,600
8	Miscellaneous including insurance, harvesting and medicine etc.	L/s			5000
	Total B				**108,075**
	Total Cost				**315,575**

Annexure -I(Contd.)

C. Production

1	Survival(%)	60%
2	Average weight at harvest (gms)	70
3	Total production (Kg)	1,260
4	Farm gate price (Rs.)	170
5	Number of Crops per annum	1
6	Income during 1st year (85% of total production)	1,82,070
	Income from 2nd year onwards	214,200

D. Financial Analysis

	1	**2**	**3**	**4**	**5**	**6**	**7**
Capital cost	207,500						
Recurring cost	108,075	108,075	108,075	108,075	108,075	108,075	108,075
Total cost	315,575	108,075	108,075	108,075	108,075	108,075	108,075
Income	182,070	214,200	214,200	214,200	214,200	214,200	214,200
New benefit	-133,505	106,125	106,125	106,125	106,125	106,125	106,125
NPW of cost	630,072						
NPW of benefit	863,222						
NPW	233,150						
BCR	1.37						
IRR	77%						

MUD CRAB FARMING

Mud crab farming is very popular in some Asian countries like Bangladesh, India, Thailand, Philippine etc. Mud crab has huge demand and price in international market. Crab is very tasty and many countries of the world import huge amount of crabs for consumption every year. As a result, there are huge possibilities of earning foreign currencies by exporting crabs. The main benefits of crab farming are, labor cost is very low, production cost is comparatively lower and they grow very fast. Commercial crab farming business is developing the lifestyle of the people of coastal areas. By proper care and management we can earn more from crab farming business than shrimp farming. And small scale crab farming is gaining popularity day by day. Mud crab farming systems in coastal areas are described below.

Types of Mud Crab

Mud crab can be found on estuaries, backwaters and coastal ares. They are member of Scylla genus. There are two species of crabs available that are suitable for commercial production. Two species of crabs are red claw and green mud crab.

Green Mud Crab

- Green mud crabs are larger in size.
- A green mud crab can grow to a maximum size of 22 centimeter carapace width. And it can weights about 2 kg.
- These are free living and distinguished by the polygonal markings present on all appendages.

Red Claw

- Generally red claws are smaller in size than green mud crab.
- A red claw can grow to a maximum size of 12.7 centimeter carapace width. And it can weights about 1.2 kg.
- It has a burrowing habit and there are no polygonal markings on it.

Both species are suitable for commercial crab farming business. And both have good value and huge demand in the foreign market.

Mud Crab Farming Methods

You can raise mud crabs in two systems. Grow out farming and fattening systems. The systems of farming in this two methods are shortly described below.

Grow Out System

In grow out farming system, young crabs are raised and grown for a certain period of 5 to 6 months till they reach marketing size and weight. This type of crab farming system is generally pond based. The pond size depends on the production type. Generally ponds for crab farming sized between 0.5 to 2 hectors. Proper bunds and tidal water exchange is a must. Small sized ponds are very suitable for crab farming. Because they are easily maintained. Make a suitable fence if the size of pond become small. In larger sized ponds where natural conditions are prevailing, strengthening is necessary along the outlet area. You can stock wild collected juvenile crabs that weights around 10 to 100 grams. Depending on the size of crabs and available facilities the duration of production may varies between 3 to 6 months. In commercial production with supplementary feeding you can stock 1-3 crabs per square meter. You can feed your crabs low cost fish, shrimps, small sized crabs etc. You can visit your nearest local market and collect rotted fish and innards of birds and animals from slaughter house. Provide the crabs 5% feed daily of their total body weight. For example, if there are 100 kg crabs in the pond then feed 5 kg food daily. Collect some crabs and try to determine an average weight. Regular sampling is very necessary for monitoring the growth and general health, and to adjust the feeding rate. Keep some pipes in the pond for shelter and the purpose of reducing mutual attacks and cannibalism. Within 3 to 5 months they will reach marketing weight and become suitable for selling.

Fattening System

Raising soft shelled crabs for a certain period until their exoskeleton gets hardened is known as crab fattening system. Hard shelled crabs has four to five times more value in the market than

soft shelled crabs. Farming crabs in this system take less time and the process is very profitable. You can do crab fattening business in two systems that are described below.

- **Fattening in Pond:** Fattening can be done in any types of ponds between 0.025 to 0.2 hector size. Small tidal ponds with a depth of 1 to 1.5 meter is very suitable for crab farming. Prepare the pond perfectly before stocking crabs in the pond. Pond preparation can be done by draining the pond water, sun-drying and adding sufficient quantity of lime. Make a fence around the pond for fattening purpose. Because the crabs have a tendency to escape by making hole and digging the soil. Reinforce the inlet areas with bamboo matting inside the bund. For stocking, collect soft crabs from local fisherman or crab merchants. Collect the crabs in morning. 1-2 per squire meter stocking density is ideal for crab fattening purpose. Divide the pond into different compartments according to the size of crabs if it is big sized. Keeping male and female crabs separated from each other will make good results and reduce mutual attacks and cannibalism. Depending on your location and crabs availability 8 to 12 fattening cycles can be done in a year. Generally, crabs weight between 300 grams to 500 grams have high demand and value in the market. Collect and sell all the crabs when they reach the marketing weight. Always try to sell the crabs when they are in hard shelled condition. This will ensure high profit form crab farming business.
- **Fattening in Pens or Cages:** Crab fattening can also be done in pens, floating net cages, bamboo cages in shallow estuarine waterways and inside large shrimp ponds with good tidal water influx and in tanks. You can use bamboo splits, netlon or HDPE as netting material. 3 m * 2 m *1 m (3 m long, 2 m wide and 1 m height) is ideal cage size for crab fattening. Arrange the cages in a row so that you can easily feed and monitor the crabs. Stocking density of 10 crabs per squire meter in cage and 5 crabs per squire meter in pens is ideal. Maximum stocking density can result mutual attacks and cannibalism. Fattening in cages or pens in only used in small sale production. For commercial production fattening in ponds is perfect and more profitable.

Between these two crab farming methods, fattening system is more profitable than grow out system and has many advantages. Grow out crab farming system takes more time than fattening system. But fattening system is very popular to the farmer as it take less time and highly profitable.

Water Quality

Water quality plays an important role in the production of crabs. Change water occasionally if possible or apply proper medicines or chemicals. See the following chart.

Salinity	15-25%
Temperature	26-30° C
Oxygen	> 3 ppm
pH	7.8-8.5

Feeding

For commercial purpose, crabs need 5-8% food of their body weight. You can feed your crabs low cost trash fish, chicken waste, animal innards collected form slaughter house, brackish water clams etc. Don't served all the feed at once. Instead give it twice a day. Give major part of the total feeds during evening hours.

Marketing

After a certain period check the crabs for their hardening. In grow out crab farming system they become suitable for marketing purpose within their 3 to 6 months of age. And in fattening system the time depends on crab's size. However, collect the crabs when they reach proper weight and when their price remain high. Collect the crabs in the early morning hours or evening hours. You can collect crabs from pond by using scoop net or by using alluring bait. Wash the collected crabs with good brackish water and remove all types of dirt and mud. And then carefully tie the crabs very carefully without breaking its legs. Then try to keep those crabs in moist conditions. Keep them away from sunlight. Because direct sunlight has a negative effect on their survival. After that send them to the market.

Commercial crab farming business is gaining popularity day by day in many coastal areas around the world. Because it is a very easy, profitable and takes less time. Mud crabs have huge demand and high value in international market. So, you can earn some extra money and make an employment opportunity by doing commercial crab farming business.

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Lobster culture

INTRODUCTION

- Eight species of spiny lobsters, six shallow water and two deep sea species, and two species of slipper or sand lobsters constitute the lobster fishery of India.
- Spiny or rock lobsters have a sub-cylindrical body with long cylindrical antenna with whip like flagellum.
- The carapace is covered with numerous spines and tubercles.
- The slipper or sand lobsters are with a dorsoventrally flattened body and short scale like antenna without whip like flagellum.

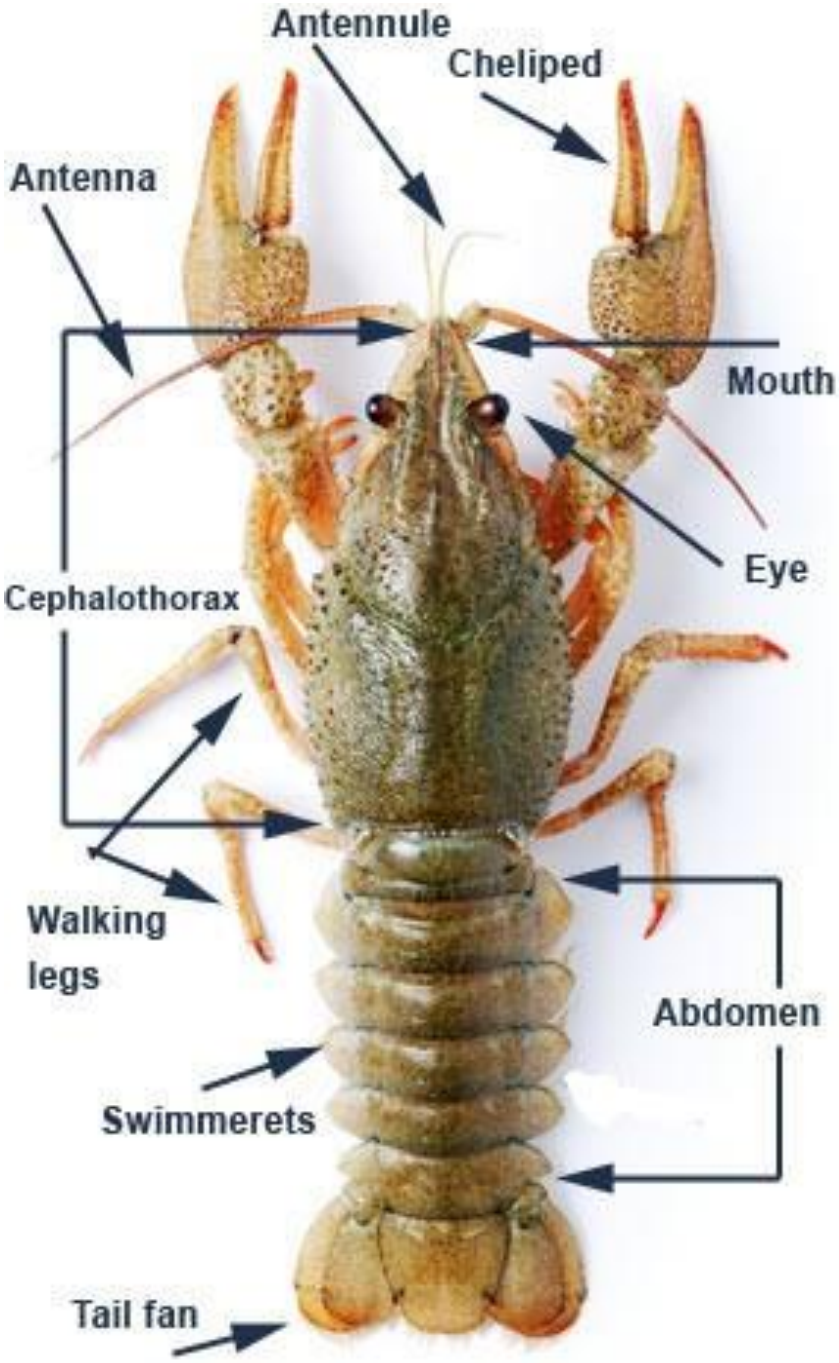

Antennule
Cheliped
Antenna
Mouth
Eye
Cephalothorax
Walking
legs
Abdomen
Swimmerets
Tail fan

Table 1: Commercially Important Lobsters of India

Lobster groups	Species	Trade name	Area of Exploitation	Level of Exploitation	Peak season	Gears used
Shallow water Spiny lobsters	*Panulirus polyphagus**	Green Lobster	west & east coasts	high	December to February	trawl net
	*Panulirus homarus**	Green Lobster	west & east coasts	high	December to March	gill net, traps
	Panulirus versicolor	Green Lobster	south & east coasts	Moderate	December to February	gill net, traps
	Panulirus ornatus	Tiger Lobster	south & east coasts	High	December to March	gill net, traps
	Panulirus longpipes	Red Lobster	south & east coasts	Low	December to March	gill net traps
	Panulirus penicillatus	Black Lobster	south & east coasts	Low	December to March	gill net, traps
Deep Sea lobster	*Puerulus sewelli*	Deep sea Lobster	west & east coasts & Andamans	High	December to April	trawl net
	Linuparus somniosus	Deep sea Lobster	west & east coasts	Low	December to April	trawl net
Sand/Slipper lobster	*Thenus orientalis*	Sand.slipper lobster	west & east coasts	High	December to January	trawl net
	Scyllarus sordidus	Sand/slipper lobster	west & east coasts	Low	December to January	trawl net

*Cultivable Species

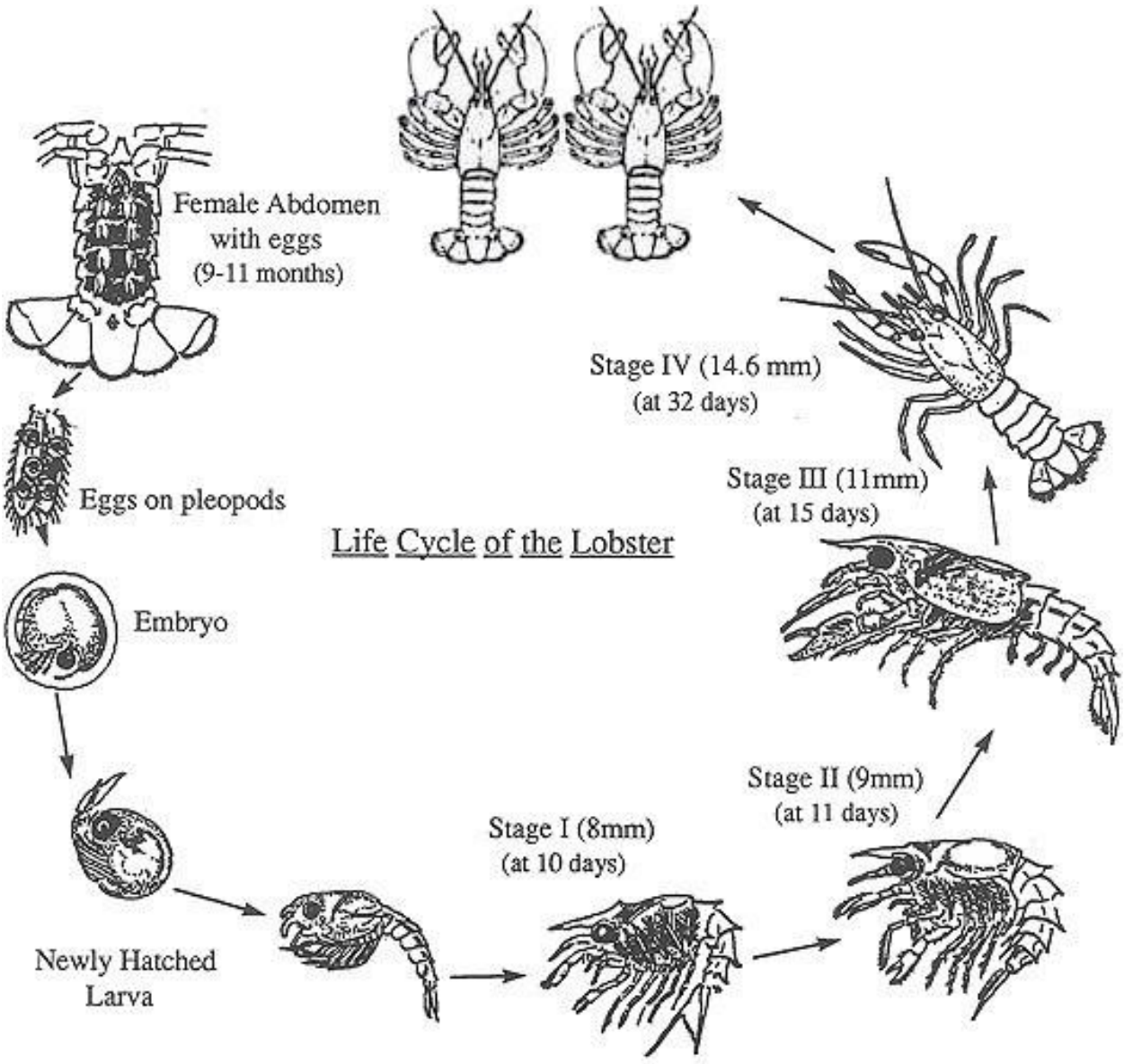
RESOURCES

- Lobster catch in India is around 2000-3000 tonnes per annum and most of it is exported frozen, whole cooked or live.
- Export of whole lobsters since late 80's and live lobsters since 1993 and the ever increasing demand for Indian lobsters have resulted in their regular and organised exploitation.
- Maharashtra and Gujarat are the main lobster fishing states followed by Tamil Nadu.

- While lobsters are landed as a bycatch in fish/shrimp trawls in the north-west coast, they are caught by gillnets, traps and occasionally by trawls in the south-east and south-west coasts
- Lobsters weighing 200 to 300g are best suited for whole cooked product while those weighing over 300g (greens) and 500g (tiger) are in demand for live lobster export.
- High demand for live lobsters, which is Rs.600-1500/kg depending on size, has recently generated considerable interest in culture/fattening of spiny lobsters.

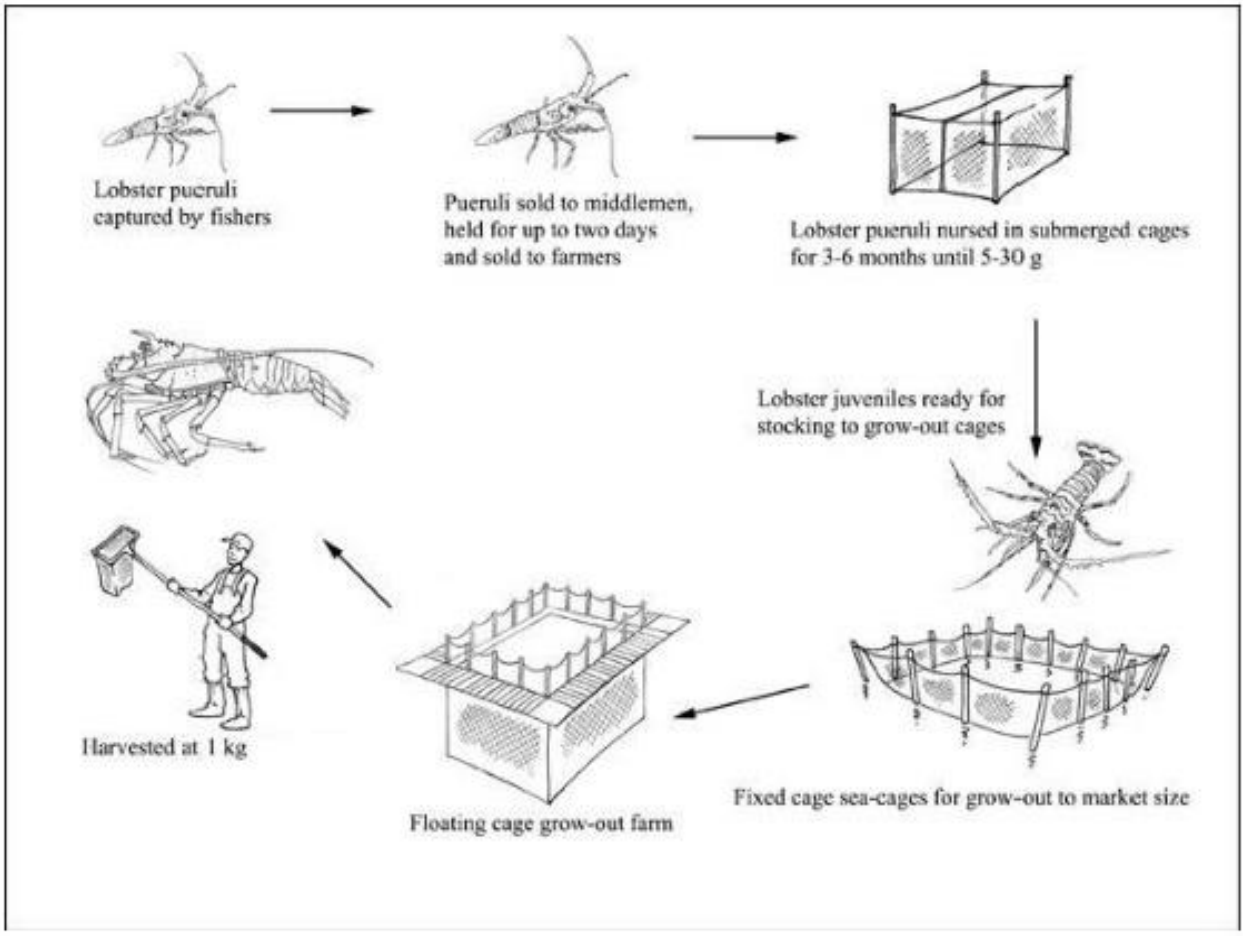
Seed

- All commercially important species of shallow water spiny lobsters in India have been bred in captivity, but their whole larval cycle is yet to be completed.
- Scientists at the Central Marine Fishers Research Institute (CMFRI) were successful in rearing lobster larvae to more than half way stage and efforts are on to complete the larval rearing process.
- In India, at present, has to start with collection of lobster juveniles from nature and growing them to the required size.
- As there are no size regulation in our country, about a third of our commercial-catch are undersized juveniles.
- These juveniles can be utilized for lobster culture/fattening.



Nursery

- The nursery phase typically involves stocking the pueruli at 50-100/m2 into submerged cages, consisting of mesh surrounding a steel frame.
- Each cage is placed on the sea floor at 2-5 m depth and a feeding tube from the surface to the cage provides the means to feed the baby lobsters.
- Finely chopped trash fish, crustaceans and molluscs are used as food.
- The nursery phase lasts for 3-6 months, during which the lobsters grow to 10-30 g.



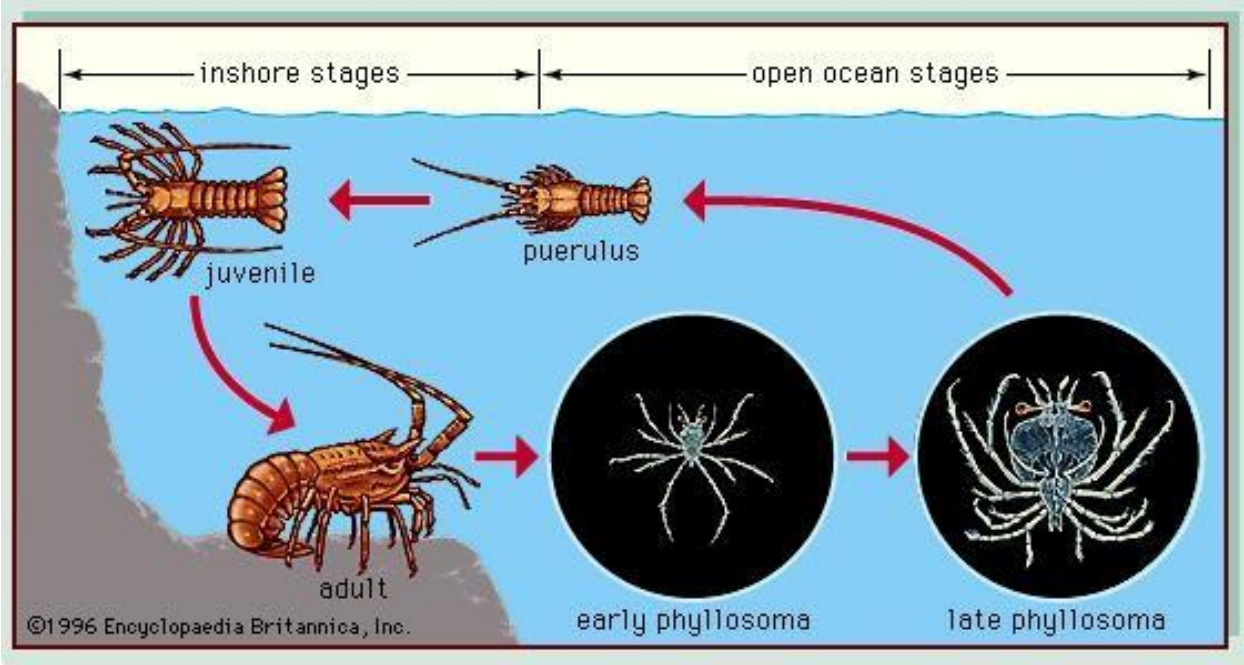
Grow-out of tropical spiny lobsters is performed in sea cages

- cages are now deployed in deep water from floating frames that are moored to the bottom.
- Grow-out cages are typically square in cross-section 3 to 4 m along each side and from 3 to 5 m deep.
- Individual farms vary in size from 10 to over 80 cages housed within one inter-linked floating framework, with narrow walkways between the cages for access.
- Each farm includes a small house for the farmer which is manned continually to ensure security of stock and to perform routine feeding, cleaning, harvesting and re-stocking.
- Lobsters are typically stocked for on-growing at 10-50 g each.
- These smaller lobsters may be stocked into cages with a smaller mesh size to ensure they do not escape.
- Stocking density may be up to 30/m².

- As lobsters grow, they are periodically harvested and manually graded to minimise the size variation within each cage.
- Larger lobsters are stocked at lower densities, typically around 5/m2 at 200 g and 2/m2 at 500 g.
- *P. ornatus* is usually on-grown to 1 kg which achieves the best price for export to China.
- This typically takes 18-20 months.
- In Indonesia, where *P. homarus* is most commonly farmed, the desired market size is 100-300 g, which takes ~9 months.

Fattening

- The tiger, *P. ornatus* is the ideal speoies due to its faster growth rate and maximum value in live export.
- *P. ornatus* of 100-150g siz can be grown to 500g in about 8 months in indoor culture systems under ideal rearing conditions.
- Since they attain maturity only at larger-size (700-800g), juveniles of this species are more suited for farming to the target size of 500g and above.



- Fattening of larger size (300-350g to 500; 750-800g to 1000g) can be done in shorter period of 3 to 4 months.

Growth enhancement by eyestalk ablation

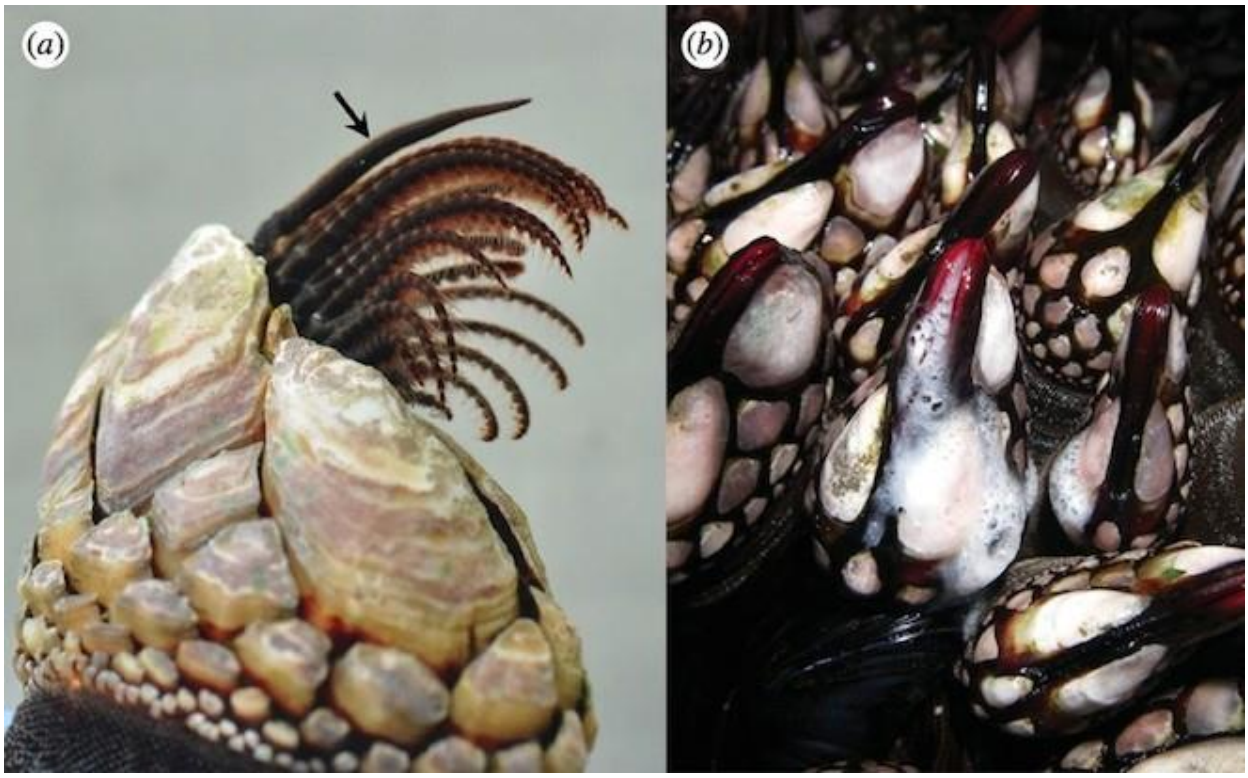
- Three to seven fold growth enhancement was achieved in four species of Indian spiny lobsters by bilateral eyestalk ablation (removal of both the eyes).
- The tiger has been grown from 100g to 1500 gm 8 months by this technique.
- Research is on to find out whether the same result can be achieved by inactivating the eyestalk hormones by laser or other modern techniques, rather than by eyestalk ablation.

Factors influencing growth of lobsters

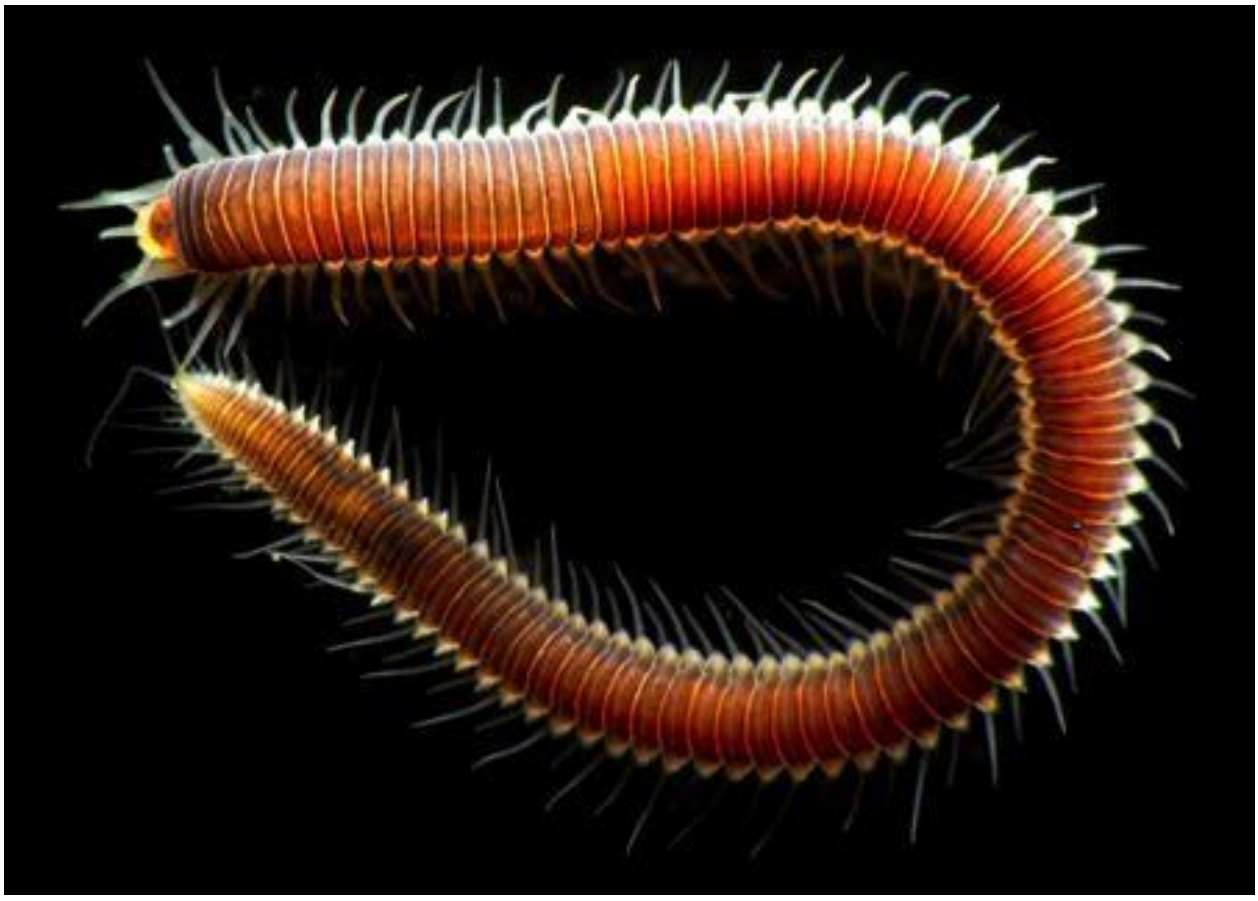
- Salinity, dissolved oxygen (DO), pH, temperature and nitrogenous metabolic wastes, especially, ammonia, are the major water quality parameters regulating lobster growth.
- Stocking density, provision of shelter, handling stress and intensity of light also influence growth in captivity.
- Quality of feed plays a major role in obtaining optimum growth and body colouration

Feed supply

- In nature, spiny lobsters feed predominantly mussels, barnacles, small crabs, echinoderms and plychaete worms
- Farmed lobsters are traditionally fed a mixture of fish, crustaceans and molluscs which come from the fish markets nearby.
- This so-called ‘trash fish’ can be highly nutritious if fresh and handled appropriately.


(a)
(b)


Starfish
Sand Dollar
Sea Cucumber
Sea Urchin



Harvesting techniques

- Lobsters are easily harvested from the sea cages by pulling the net cage to the surface and retrieving the lobsters by hand.
- Lobsters ready for market are placed in styrofoam boxes and returned to shore to processing / export facilities.
- The farmer typically sells the lobsters at this point and the wholesaler takes on the responsibility for further handling and transport to market.

Live export of lobsters

- Live lobster export, started in 1993, touched 24 tonnes in 1994 and is on the upward trend reaching 99 tonnes in 1996.
- Madras is the main city for live lobster export with a share of more than 90%.
- Bombay and Thiruvananthapuram are the other cities from where lobsters are exported live.

Handling and processing

- Wholesalers and exporters of farmed spiny lobsters employ live holding systems, consisting of tanks with clean seawater that usually involve recirculation technology to maintain high water quality.
- Lobsters purchased from the farmers are held only briefly for 1 or 2 days to maximise their quality and are generally not fed.
- They may be cooled to 10-15 ºC to slow their metabolism and improve survivability during transport. Individual lobsters are normally wrapped in newspaper and placed in styrofoam boxes before being air freighted to market.

Production costs

- Tropical spiny lobster farming is currently (2010) a profitable business with moderate to high establishment and operating costs and high returns.
- In Vietnam the cost-benefit ratio is around 1.4 and average net revenue around USD 15 000/yr per farm.
- The most significant operating cost is feed, which accounts for more than 60 per cent.
- The cost of lobster seed is also significant (22 percent).

- *P. ornatus* harvested at 1 kg are sold at about USD 45-60/kg in Vietnam.
- In Indonesia, *P. homarus* harvested at 100-300 g fetch USD 30-40/kg.

EDIBLE OYSTER CULTURE

Marine animals belonging to the families *Ostreidae* are called oysters in common usage. Oyster is one of the best known and most widely cultivated marine animals.The oysters are highly esteemed sea food and considered a delicacy in USA, Europe, Japan etc.

In India there is a growing demand for oyster meat in some parts of the country. Until recently, oyster farming has been considered as a traditional practice followed only in the temperate countries. The awareness about the vast potentialities for development of oyster farming in tropics is recent.Serious efforts are now being directed in its development under tropical conditions.

Scope for oyster farming in India

- In India pioneering attempts were made by James Hornell in 1910 in developing Oyster culture in erstwhile Madras state. Central Marine Fisheries Research Institute undertook scientific investigations at Tuticorin from early 70's and as a result, complete package of the technology is now available in the country. Vast stretches of backwaters, estuaries and bays present along Indian coast harbour natural population of the oyster suggesting suitability of the habitat for oyster culture. Being filter feeders, the oyster converts primary production in the water into nutritious sea food.

Candidate species

Six species of oysters namely the

- Indian backwater oyster *Crassostrea madrasensis,*
- Chinese oyster, *C.rivularis,*
- West coast oyster, *C.gryphoides,*
- Indian rock oyster, *Saccostrea cucullata,*
- Bombay Oyster, *Saxostrea cucullata,* and
- Giant oyster *Hyostissa hyotis* are found in India.

The first four species mentioned above are of commercial value.

Of the six species of oysters. The Indian backwater oyster *C. madrasensis* is the dominant species, more widely distributed, is euryhaline and inhabits backwaters, creeks, bays and lagoons and occurs in the coastal areas of the States of Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Andamans. *C.gryphoides* is also euryhaline and occurs along north Karnataka, Goa and Maharashtra coast. C.rivularis is found along Gujarat and Maharashtra coast while *Saccostrea cucullata*is found all along the main land coast and Andamans and Lakshadweep islands.

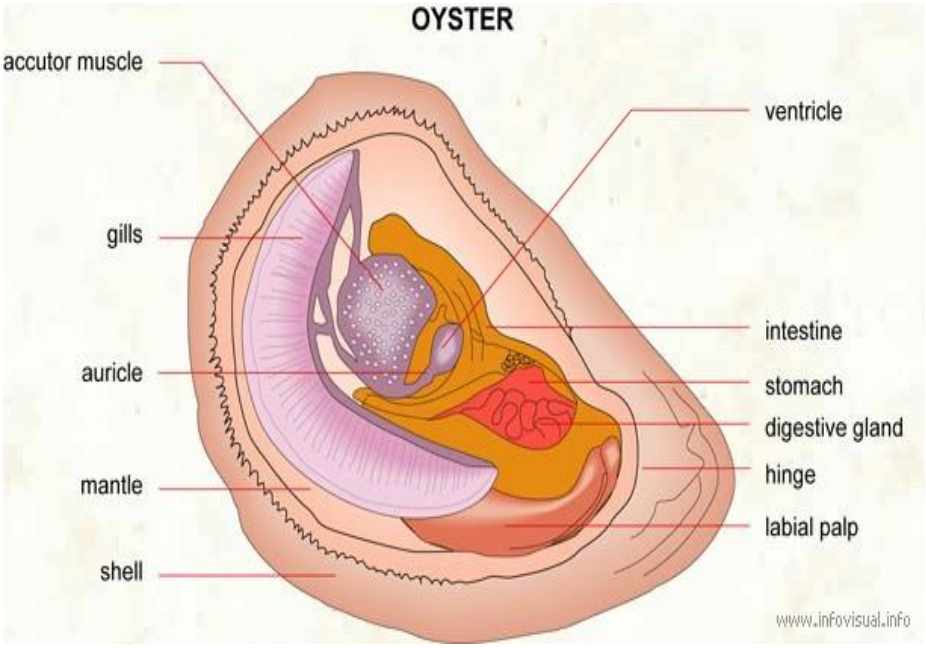
BIOLOGY, TECHNICAL PARAMETERS AND FARMING PRACTICES OF *Crassostrea madrasensis*

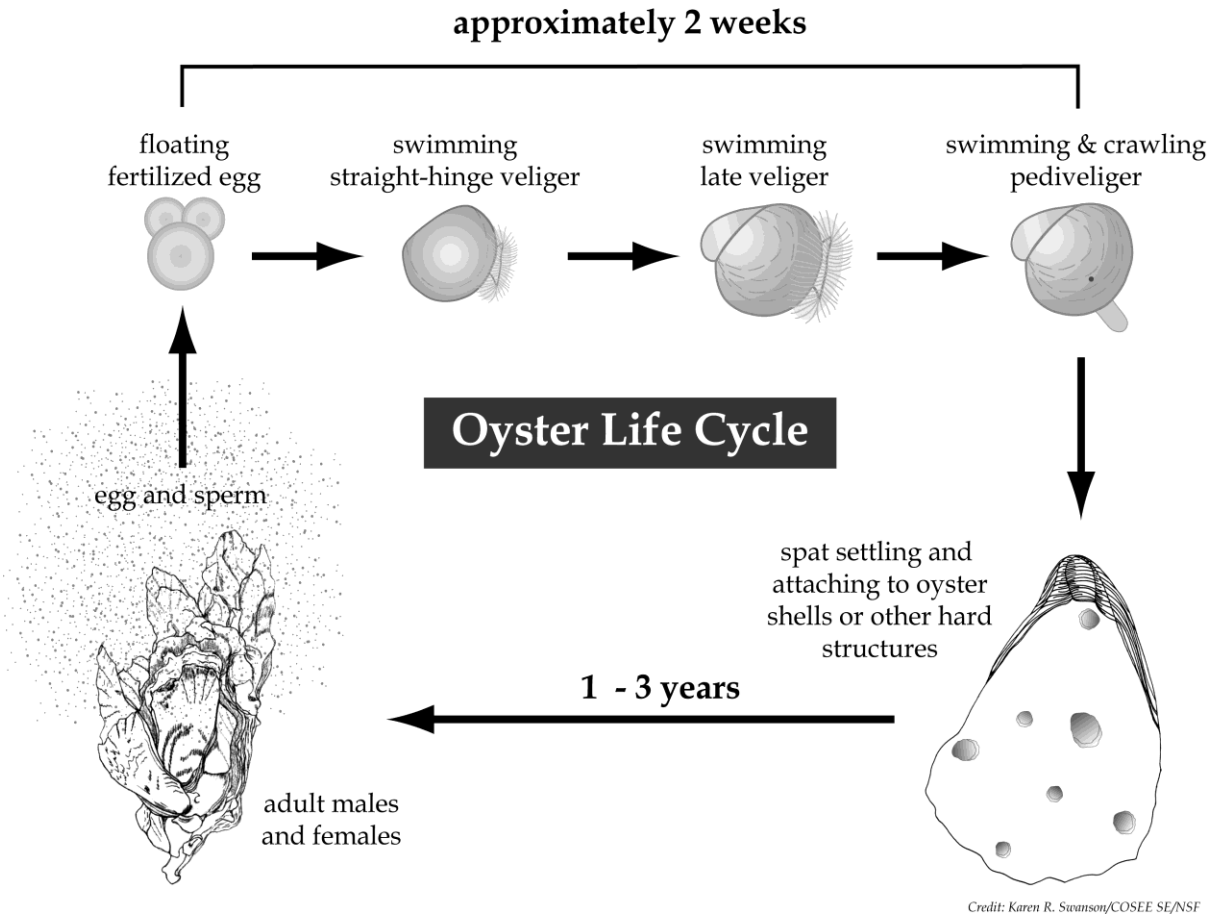
(I) Biology of *C.madrasensis*

The edible oyster is a sedentary animal. The soft body of the animal is encased in two shell valves out of which the upper valve acts as a lid to open and close by contraction and relaxation of the adductor muscle. Oysters mainly feed on organic detritus and phytoplanktonic organisms like diatoms and nanoplanktons. They are also capable of absorbing dissolved organic matter in the water through the surface of gills, palps and the mantle.

Oysters are generally dioecious but hermaphrodites are not uncommon. Young oysters of *C.madrasensis,* primarily function as males (60-75 per cent) and later become females. In zero age group upto 78 mm. in length, 75 per cent are males and in one year and above with 80 - 115.5 mm. length, females represent 72 per cent. The peak spawning period is reported to be during March-April and July-September. Oysters, like other bivalve molluscs, spend the first few weeks of their lives as small, drifting larvae.When the larva is about one-third millimetre long, it attaches to a substrate (sets) undergoes a change in its internal

organs, eventually reaches sexual maturity and spawns, thus completing its life cycle.





Technology of oyster culture

The technology of oyster culture consists of two important phases namely

(A) Oyster seed production/Spat collection and

(B) Grow- out.

(A) Oyster seed production/ spat collection

The seed requirement for culture of oyster is met either from natural spat collection or through hatchery rearing. For collection of spat from natural grounds, suitable spat collectors or cultch materials are provided at appropriate time which may be oyster shells, coconut shells, asbestos sheets, mussel shells or other materials. These are arranged on Nylon rope as strings and suspended from racks in the water at suitable spots. The larval period of *C.madrasensis* is 15 to 20 days and as such exposure of collectors will be ideal just after a week or 10 days of spawning activity.

A reliable source offering sufficient quantities of spat of the desired species is critical to successful oyster culture.Natural collection is the most important source of spat and will continue to be so until commercial hatcheries are establishedMass production of oyster seed is also possible in hatchery system for which technology is available, though no commercial hatcheries are available yet. For efficient spat collection the farmer should know the

(a) spat setting season and

(b) the sites to collect sufficient spat for stocking in the grow-out ponds.

(B) Grow out

Site Selection

For selecting suitable site for farming, several factors like water depth, bottom characteristics, protection from wave action, tidal flow and height, turbidity, water quality including chemical parameters, predation, fouling, pollution and accessibility are considered. Selected areas should be sheltered from strong wave action, salinity should be from 22 to 35 ppt and temperature range should be from 21 to 31 degree Celsius.

Farming methods

- Farming methods are normally grouped as

  (a) bottom culture and

  (b) off bottom culture.

Raft, rack, long-line and stakes are used in various off-bottom culture practices.

The bottom culture method is yet to be experimented in India.

The off bottom culture methods are advantageous over the bottom culture due to the following reasons :

(i) The growth and meat yield is relatively better.
(ii) It facilitates three dimensional utilization of the culture area.
(iii) Biological functions like filtration, feeding etc. become independent of tidal flow.
(iv) Silting and predatory problems are minimum.

Various off bottom culture methods are as follows.

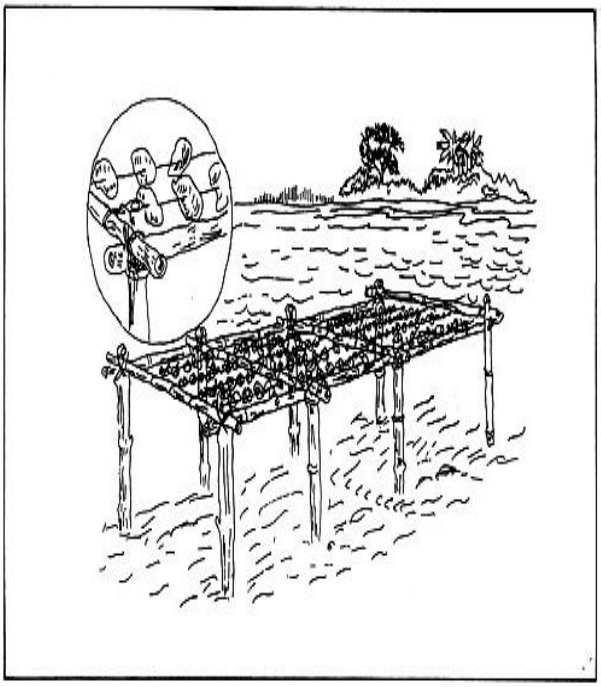
a. Rack and string (ren) method

- It is also called **ren** method. This is the most common method advocated for Indian conditions for which the oyster shell ren is used as spat collector This method is ideal for shallow estuaries, bays and backwaters. The racks are constructed at 1 to 1.25 m depth. Rack is a fixed structure, comprising several wooden poles vertically driven into the substratum over which a wooden frame is made at a height of 0.5 m, above the water level. The shell strings are suspended from these racks. A rack covering 80 m^2. area holds 90 strings and 125 racks in a ha. At the end of 7-10 months, each string may weigh 7 to 7.5 kg. and the production of oyster is estimated at 80 tons / ha. The mortality is about 45 per cent. The meat yield is about 10%.

b. Rack and tray method

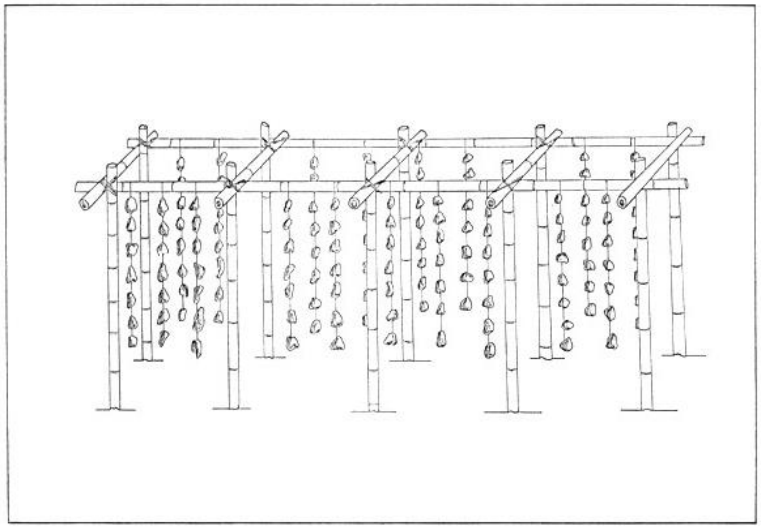
The nursery reared single spat (cultch-free) measuring about 25 mm are transferred to trays of size 40 x 40 x 10 cm at a density of 150 to 200 spat / tray. The tray is knitted with 2 mm synthetic twine of appropriate mesh size and is suspended from the rack. Once the oyster reaches 50 mm length they are segregated and transferred to rectangular tray of size 90 x 60 x 15 cm and these trays are placed on the rack which occupies 25 sq.m area and holds 150-200 oysters. The average growth rate of oyster is 7 mm/ month and at the end of 12 months, the oyster attains an average length of 85 mm.

The production estimated is 120 tons/ ha/ year which when compared to string method is higher, however the production cost is quite high.



c. Stake culture

In this culture method, stakes with one nail on the top end and two nails on the sides are driven into the substratum . These nails hold the shells with spat. The stakes are placed 60 cm. apart. In this method, the nursery rearing of spat is carried on the same stake.Initially for 2 months, the spat is covered with velon screen till a size of 25-30 mm. is attained and in another 10 months they reach marketable size.The production is estimated to be 20tonnes/ ha/ year.



d. Raft culture

Raft is the most suitable farm structure in sheltered bays where the depth is 5m and more.Wooden poles placed parallelly and tied across with coir rope to make a rigid frame.Four empty airtight barrels of about 200 litre capacity are tied to the underside of the raft at corners. It is moored by two anchors and a chain. The size of the raft varies however rafts of size 6m x 5 m. are found to be quite suitable. PVC pipes instead of wooden poles and styrofoam floats in place of barrels may be used.However, this method has not been tried in India so far.

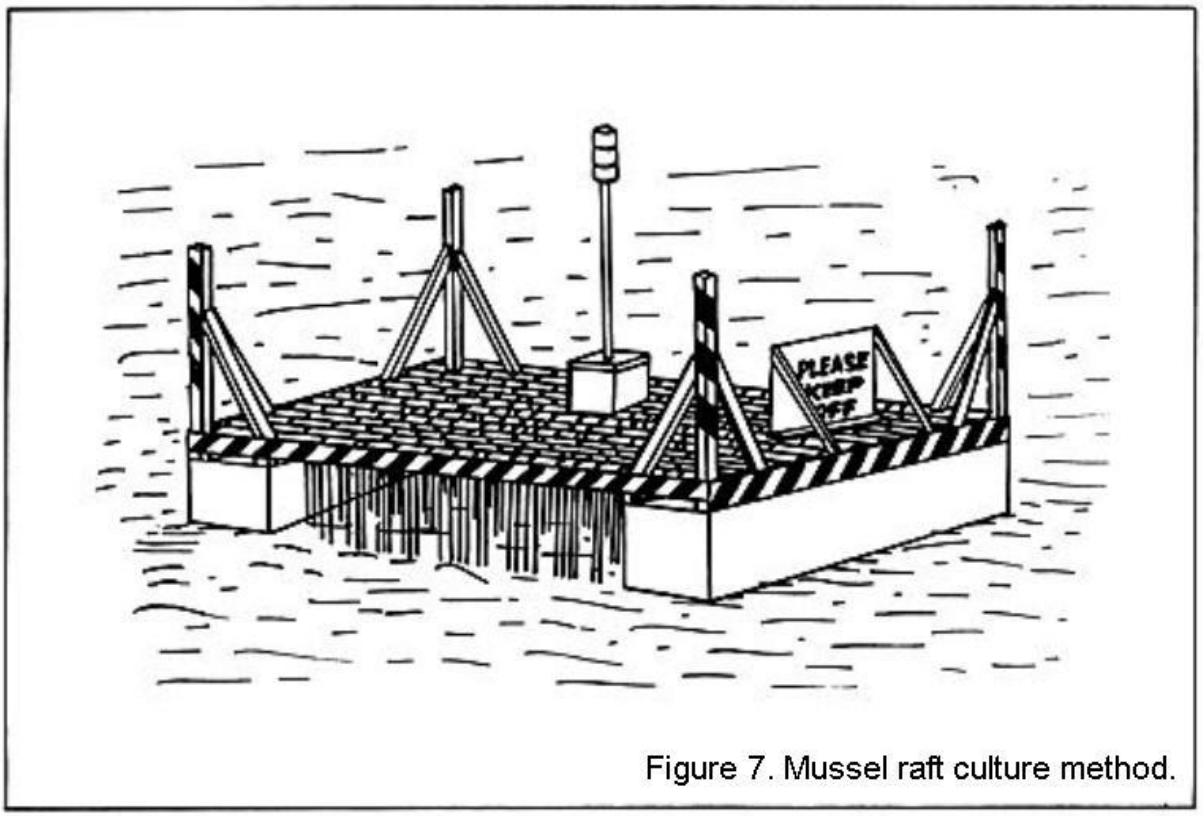
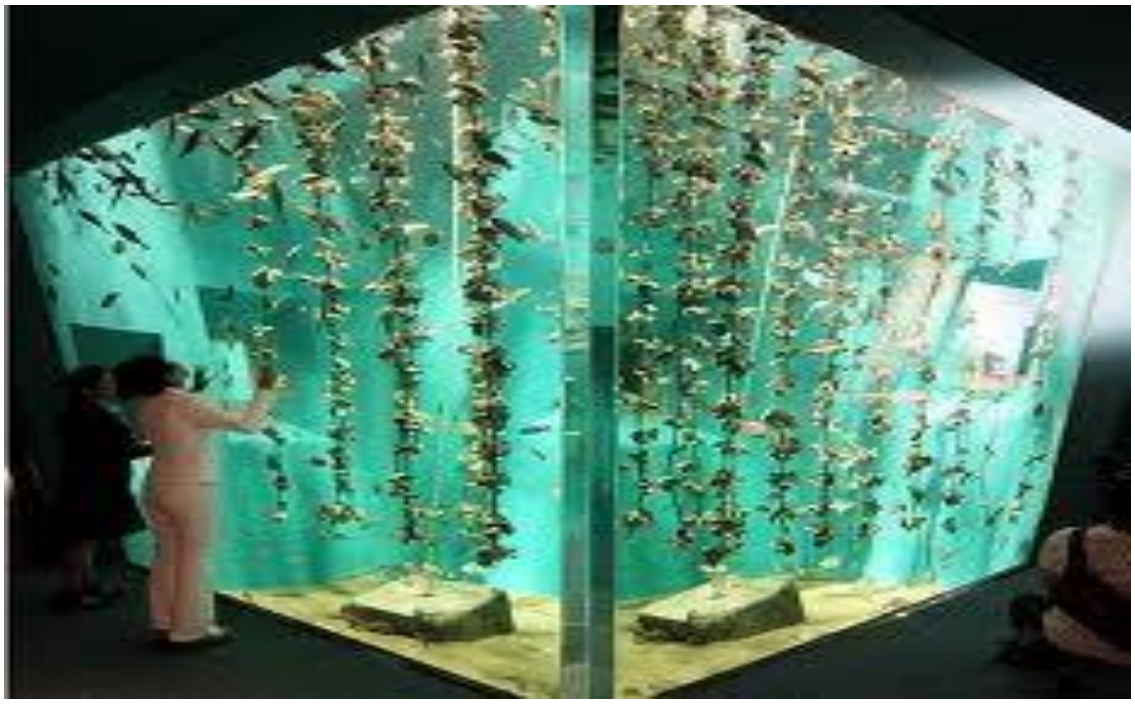


Figure 7. Mussel raft culture method.



e. Long line culture

In this system long ropes or cables are anchored at each end and are supported at intervals by floats. Long lines of 50-100 m length are easy to manage. Double long lines comprising of one line on either side of the floats are also used.

Farm management

Farm management practices involve periodic cleaning of the oyster, oyster rearing trays, farm structure like racks, thinning, sorting or grading and manual removal of predators and foulers. ‘Fouling’ includes mud, ascidians, coral, sponges and other encrusting organisms.

These agents attach themselves to trays and oysters and interfere with the feeding and respiration of the oysters. If not attended to on time, a thick blanket of fouling organisms and silt develops and the growth of oysters is hampered. Mortality also increases due to the restriction of water circulation over the animals.

Harvesting

Oysters are harvested when the condition of the meat reaches high value which in case of *C. madrasensis* is found to be good during March-April and August-September.Harvesting is done manually and oysters are transported to shore in dinghies. After landing, the harvested

oysters should be brushed and any fouling organisms removed.Oysters should be depurated to ensure they are free of bacterial contamination.

Depuration should be carried out for 36 hours. Un depurated oysters are unsafe for consumption and may cause gastroenteritis and related diseases. Reservoir water in the depuration unit should be replaced for each run. Oysters are marketed after depuration. Some oysters may be sold as shucked meat.A special ‘shucking’ knife should be used to open the oysters and remove the meat. Care must be taken not to damage the oyster meat during shucking. The meat should be weighed and then kept on ice until sale.

Training and Extension

The Central Marine Fisheries Research Institute (CMFRI), ICAR provides technology, training and extension services to the interested farmers to take up culture.

CHROMOSOMAL MANIPULATION

Chromosomal set manipulation can be used to produce highly inbred fish in a relatively short period. Individual fish with F = 100% can be produced in a single generation, while inbred lines where all fish have F = 100% can be produced in two generations. Chromosomal set manipulation to produce inbred fish can be done in one of two basic ways, but regardless of the technique used, the fish that are produced have only a single parent.

The first technique is to prevent the first mitotic division that occurs when the zygote nucleus and zygote itself divides to become a two-celled embryo.To create inbred fish, this technique is done with haploid zygotes. This technique is called either “mitotic gynogenesis” or “mitotic androgenesis,” depending on the whether the haploid set of chromosomes of the zygote comes from the mother or from the father.

The second technique is to prevent equational division (second meiotic division) of the secondary oocyte (egg) after sperm penetration; this prevents the second polar body from leaving the egg. This technique is called “meiotic gynogenesis”, because chromosomal set manipulation is accomplished by disrupting a meiotic division; it produces fish called “meiotic gynogens”, because all chromosomes in the offspring come from the mother.

This technology requires highly skilled labour, and the methodologies have not been perfected. At present, these breeding programmes are important for some kinds of genetic research, but their practical use has not been quantified. Consequently, these breeding programmes should be done only by scientists who work at agribusinesses or research institutions that are capable of conducting sophisticated genetics experiments.

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PEARL OYSTER FARMING

Taxonomy

- **Genus: *Pinctada***
  - **Includes most of the pearls found in fashion.**
- ***Pinctada maxima***
- ***Pinctada fucata***
  - **Akoya pearls (classic)**
  - ***Pinctada margartifera***
  - **Tahitian peals (black)**

– **South Sea pearls**

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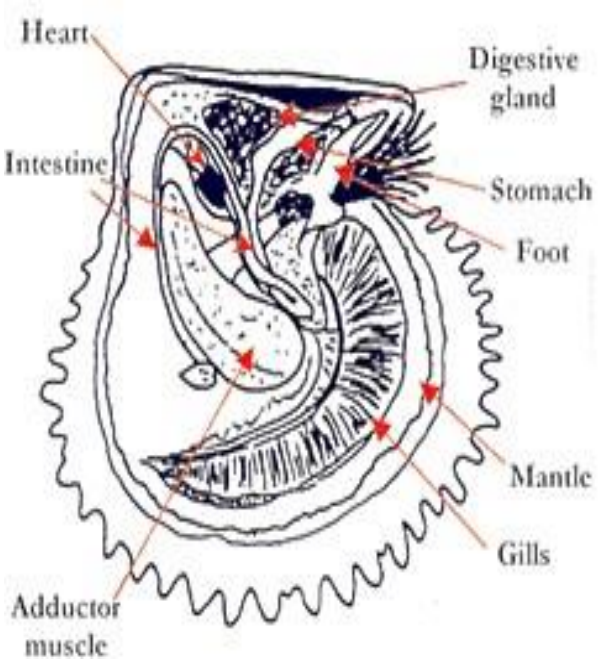


 South Sea Pearls	 Black Tahitian Pearls	 Akoya	 Freshwater Pearls	 Keshi or Poppy Seed Pearls
Saltwater oysters from Northern Australia and Southeast Asia	Black-lipped oysters from the warm waters of the South Seas	Saltwater oysters from China and Japan	Freshwater mussels primarily from China	Salt or fresh water
9 mm to 18 mm	8 mm to 15 mm	3 mm to 10 mm	2 mm to 16 mm	Extremely small and not fully formed
Silver, gold, and white	Dark hues of black, grey, silver, green, blue, and purple	White or cream with rose, cream, or ivory overtones	Pale shades of white, black, pink, peach, lavender, plum, purple, and tangerine	Various; can be dyed
Round, near round and button	All	Round, semi-round, baroque, drop	Oval, button, and baroque—but rarely round or semi-round	Various
The most expensive pearl on the market due to its rarity and luster	The only type of pearl to achieve its black color naturally	Used in the classic white pearl necklace, these typically have the greatest shine and highest luster of all cultured pearls	Not as lustrous as other types, they offer the widest range of options for pearl buyers in terms of size, shape, and color	Typically used in commercial and fashion jewelry, they are formed when the oyster rejects and spits out the implanted nucleus material before culturing is complete.

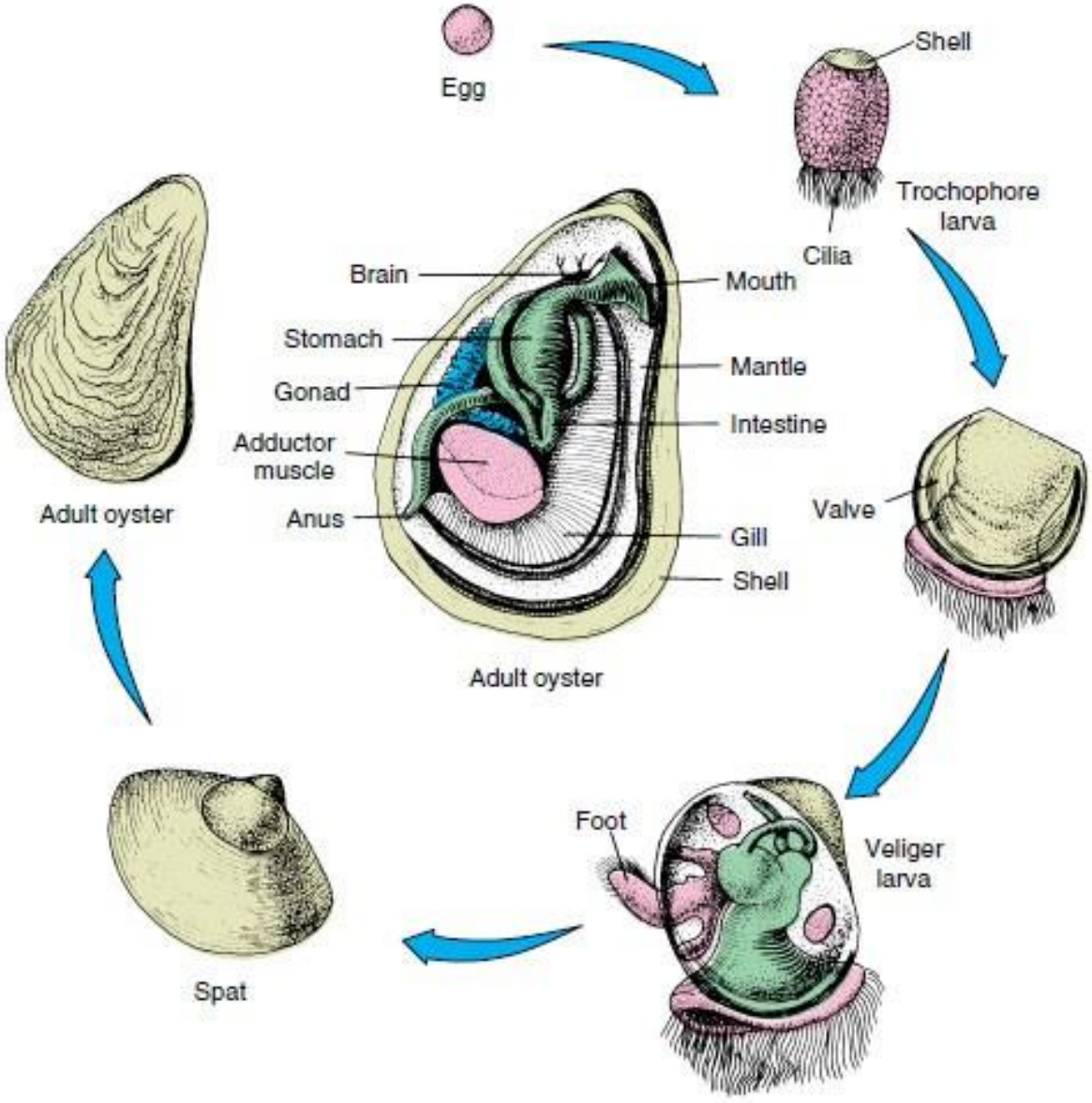
Economic Importance

- **Billion dollar retail industry**
  - **Sold all over the world**
- **Price depends on rarity and quality**
  - **$50 Pair of freshwater pearl earings to $100,000 strand of South Sea pearls.**

Reproduction in Captivity

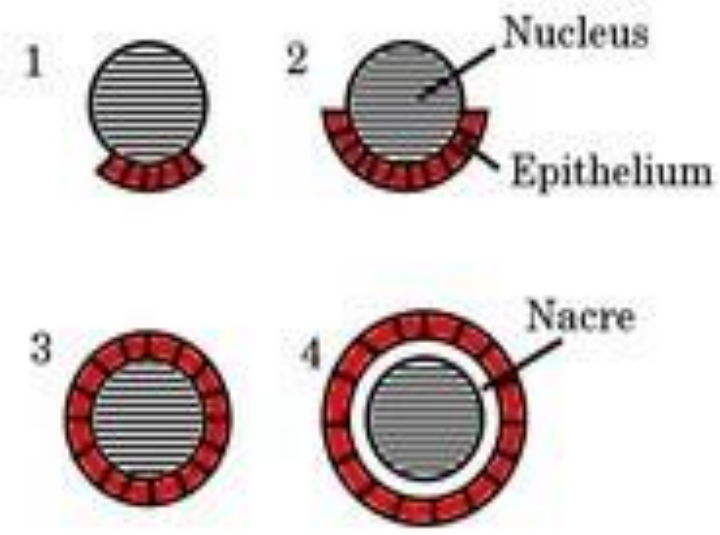
Thermal stimulation induces spawning.Larvae are allowed to float freely in the water under controlled conditions until they are a few weeks old.Once the larvae develop into baby

oysters they are moved to a “nursery” area.Remain in nursery for about 1-2 years, until they are large enough to be grafted.

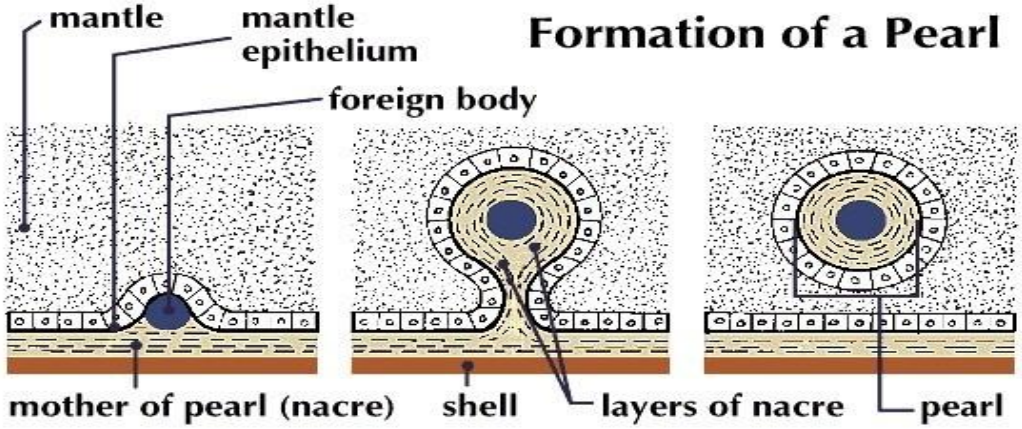


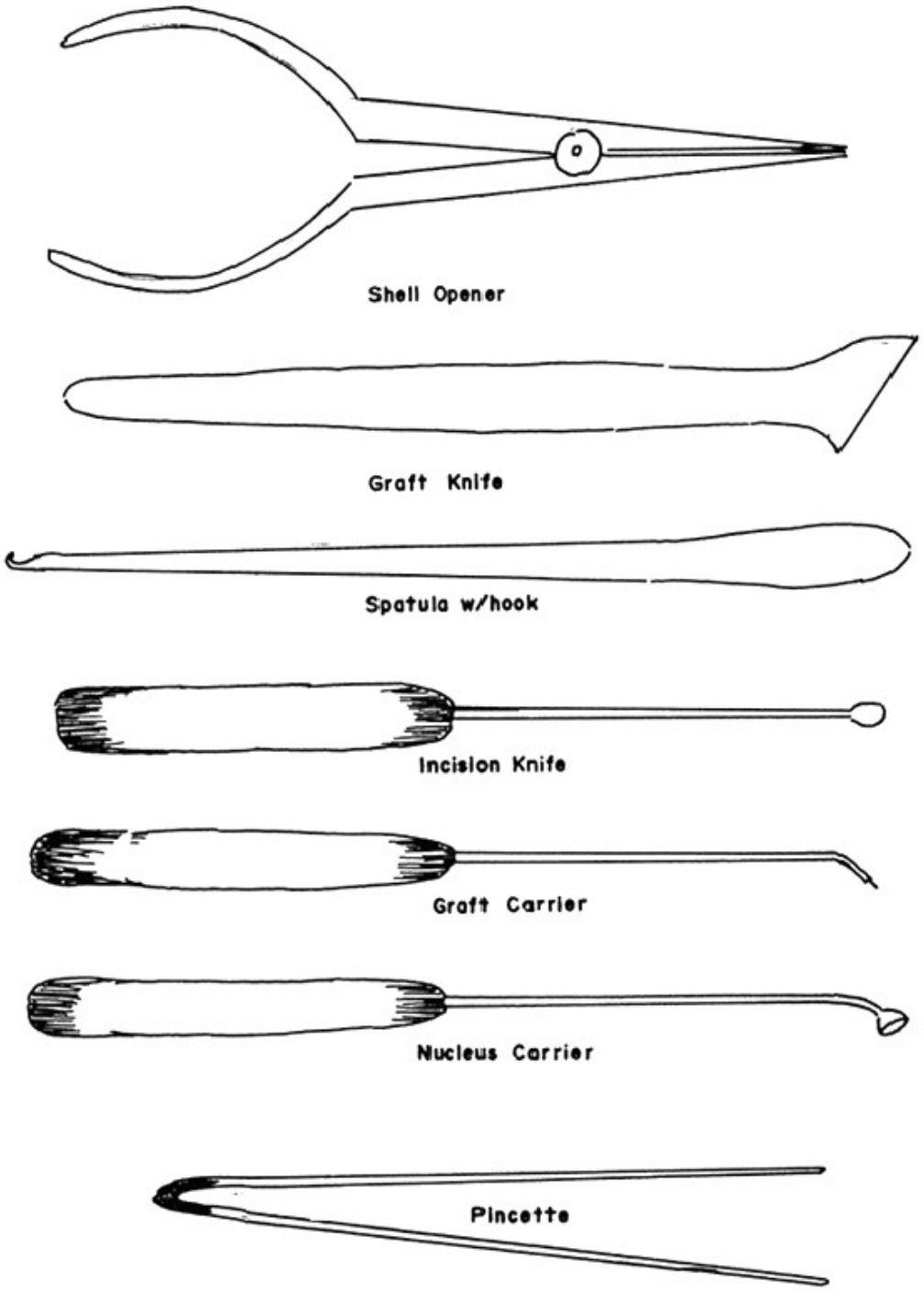
Saltwater Nucleation In Pearl Farming (Grafting)

Two basic methods of nucleation are used. Saltwater oysters are generally nucleated using a "bead", prepared from mother-of-pearl. First, the bead is surrounded by a small piece of mantle tissue taken from a donor oyster. The bead and tissue are then implanted into the oyster's gonad.



The bead serves as a mold, or nucleus, around which the pearl develops. The resulting pearl will contain the bead at its center and will tend to develop in the same general shape as the original bead. The bead can be detected in the final pearl by x-rays.





Growing

- **Raft Culturing**
  - **Appropriate for sheltered bays**

- **Long-line culture method**

  Cages are hung from horizontal ropes or chains connected to floats.Oysters are threaded at onto a small thread or rope that is hung from a raft.Good for open ocean environments

- **On-bottom culture**

  Can only be used in areas of granite or coral sand composition of the sea bottom.

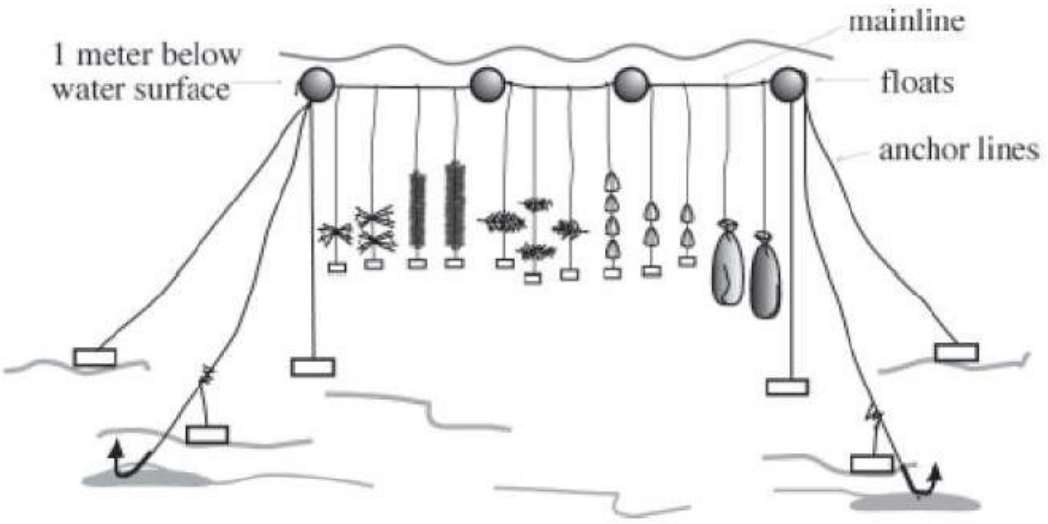
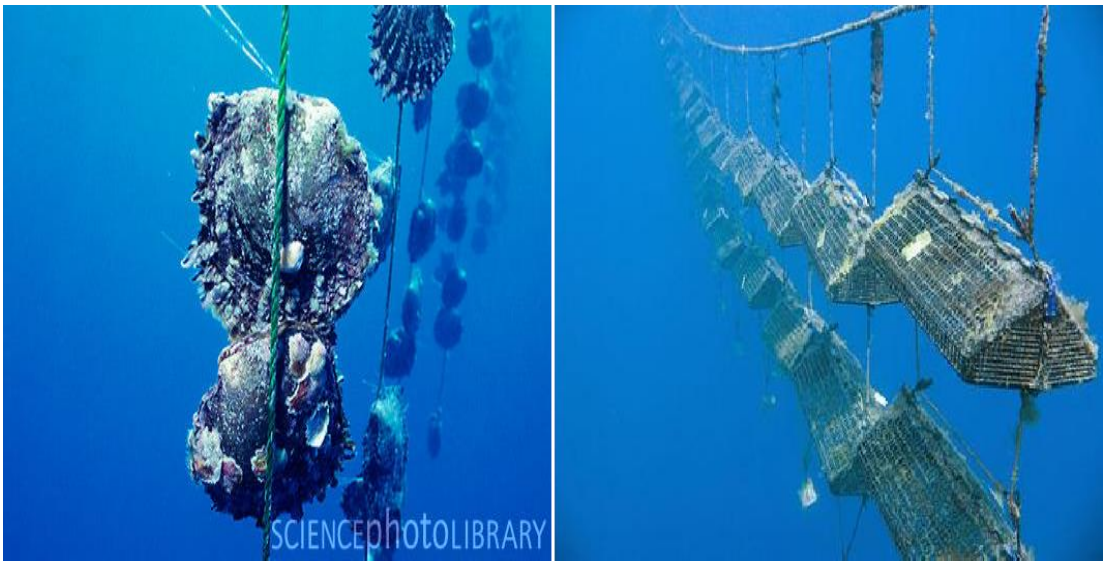
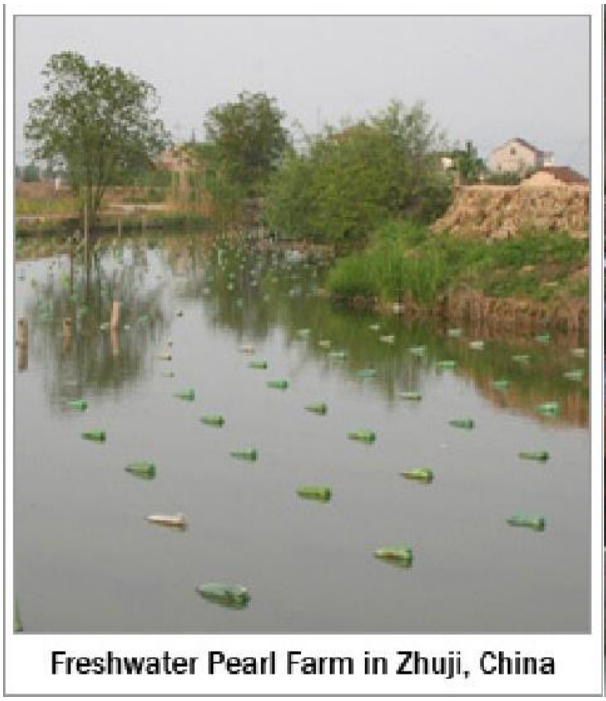


Figure 7. "Tahitian" long line. A mainline is hung from a series of anchor lines kept suspended by floats interspersed along the line. Longlines can be used to hang chaplets, spat collectors or pocket panels. Modified from Gervis and Sims (1992).


SCIENCEphotoLIBRARY

THE PEARL IS NOW ALLOWED TO GROW

After nucleating, the oysters are given a few weeks to recover from the surgery. During this time, some of the oysters may reject and expel the implanted nuclei; others may become sick or even die. Most, however, will fully recover. The oysters are then placed in cages or nets and moved into the oyster bed, where they will be tended as the pearls develop.Depending on the type of oyster, this process can require anywhere from a few additional months to several more years!



Freshwater Pearl Farm in Zhuji, China

Harvest

Akoya pearls are harvested after 8 months – 2 years. All other pearls are harvested after 2 –6 years. Harvesting is done in the winter months, when the pearl luster is highest. An x-ray can be used to determine pearl size before harvest.

After the pearls are extracted from the oysters, they are washed, dried, and sorted into general categories. Sometimes, the pearls are polished by tumbling in salt and water.The pearls are then sold to jewelers, manufacturers, and pearl dealers.

MUSSEL CULTURE

INTRODUCTION

Mussels are bivalve molluscs and are found attached to rocks or any other hard substratum by means of byssus thread secreted by the body. They belong to the family MytilidaeIn India two species of marine mussels namely *Perna viridis* the Green mussel and *Perna indica* the Brown mussel forms the major part of the fishery.

Kerala State can be called as the Mussel fishery zone of India since extensive beds of both the green and brown mussel occur in this state which also account for the bulk of mussel production in India. Of the two species commercially important the green mussel P. viridis is widely distributed and found in the beds of Chilka lake, Visakhapatnam, Kakinada, Madras, Pondichery, Cuddalore and Porto Nova on the East coast and extensively around Quilon, Alleppey, Cochin, Calicut to Kasargod, Manglore, Karwar, Goa, Malwan, Ratnagiri and the Gulf of Kutch on the West coast.

P. viridis occurs from the inter tidal zone to a depth of 15 m. On the other hand , P indica has restricted distribution and is found along the southwest coast from Varkala near Quilon to Kanyakumari and from there to Tiruchendur along the southeast coast. It occurs from the inter tidal zone to 10 m depth.P.viridis is widely distributed and hence more suitable for farming.

Perna virisis

Perna indica

1. Area suitable for farming

For sea farming, coastal waters beyond surf zone at 10 - 15 mt depth is normally selected.The area should be sheltered from strong wave action. The site should be free from any major industrial effluent and should not interfere with transport or any other fishing activity. Clear water with good phytoplankton production and moderate current to bring in the food and carry away waste products is required. A salinity range of 30-35 ppt is preferred.

Mussel farm

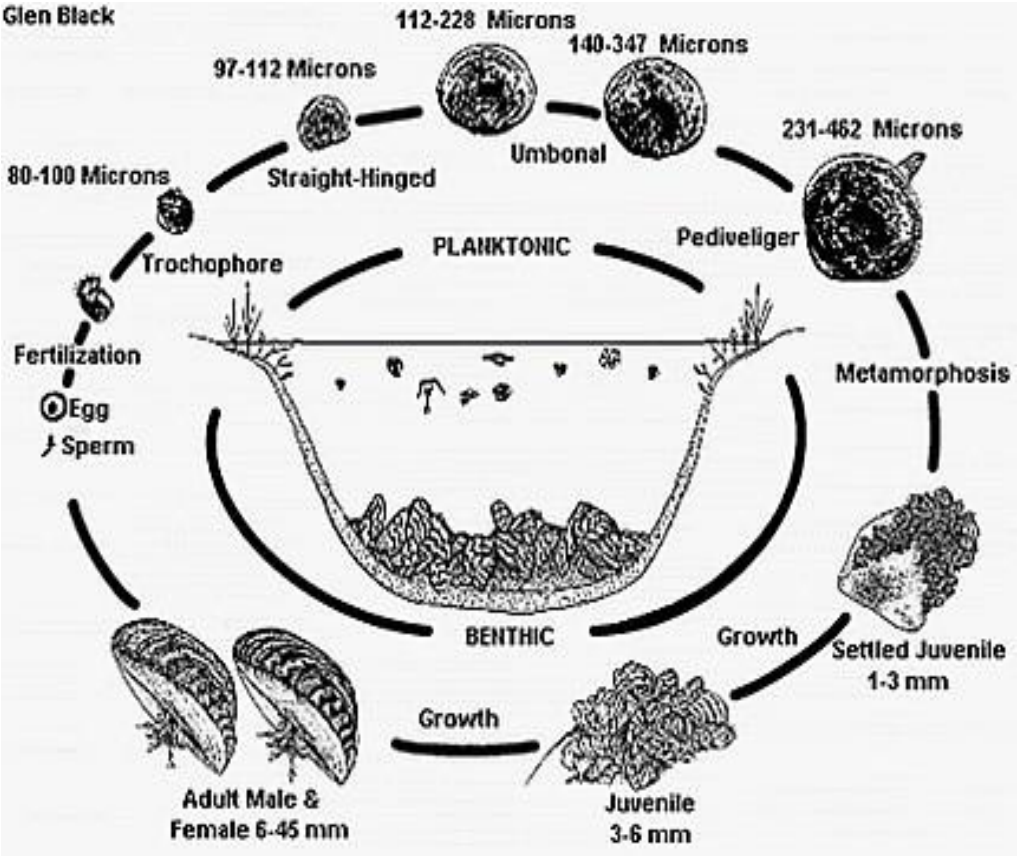
2. Farming Technology of Green Mussel

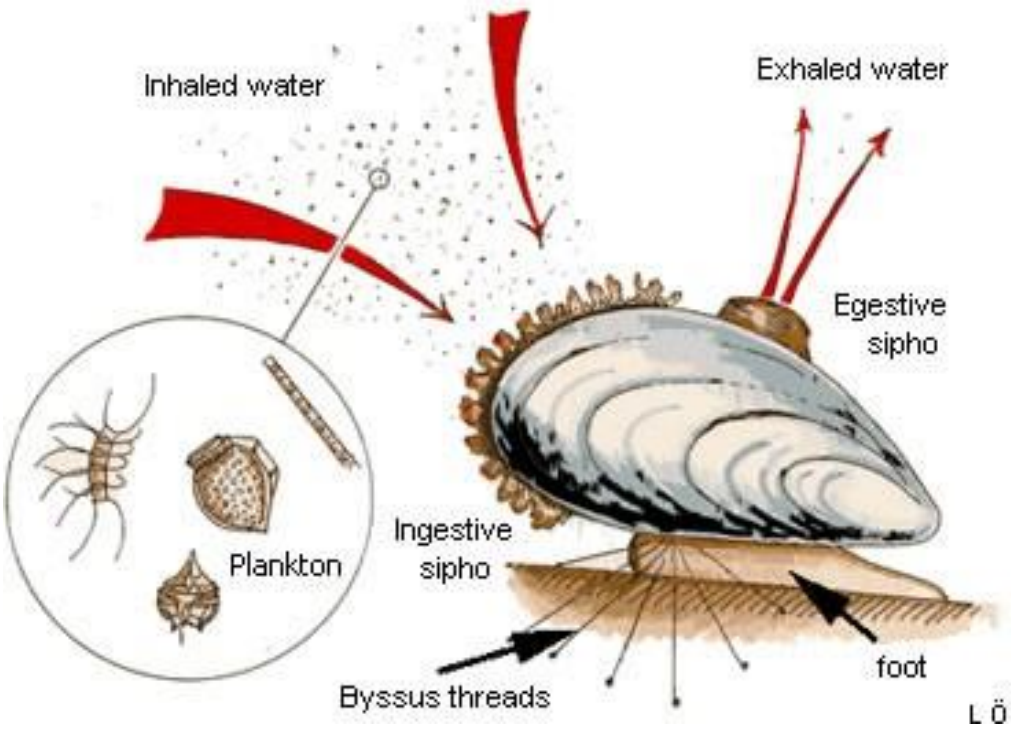
1. Biology

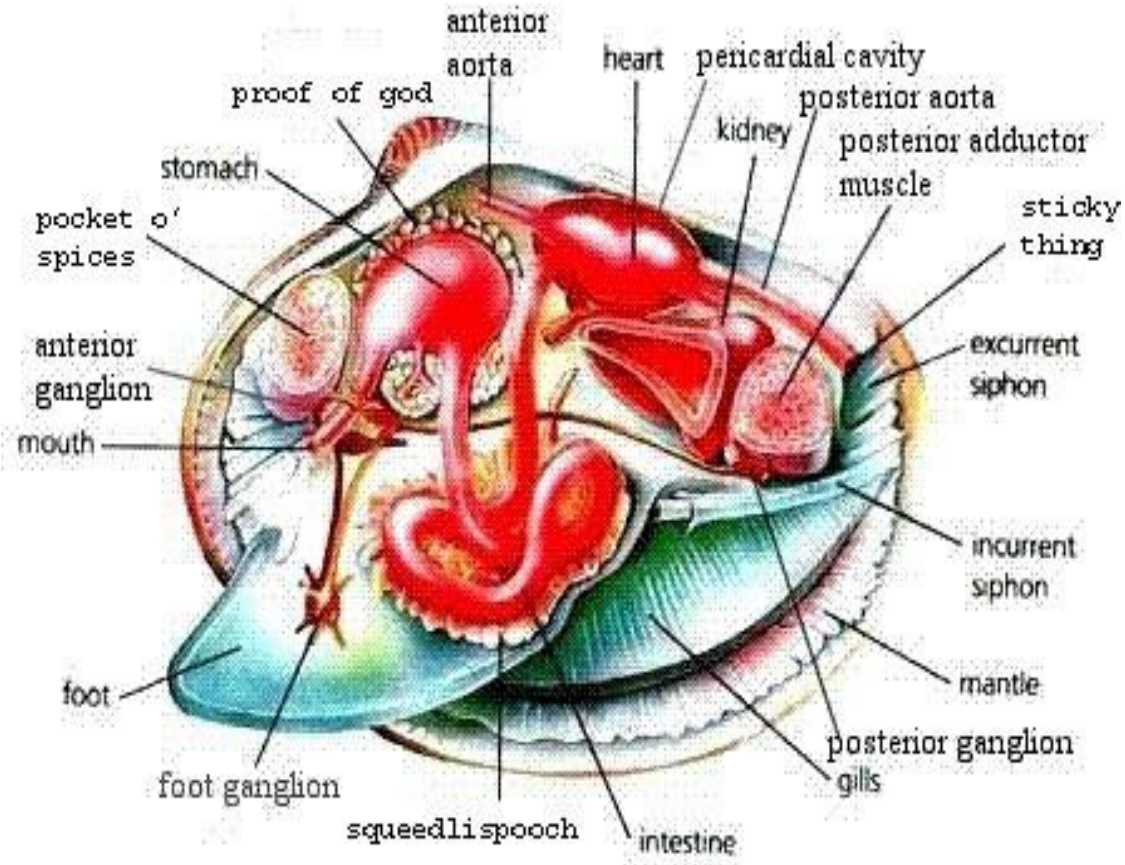
The scientific name of the green mussel is *Perna viridis*. The mussel has organ systems similar to those found in oysters with some modifications.It has a foot as in clams though smaller in size, providing limited mobility. A mussel can discard the byssal strands and secrete new ones for enabling it to change position. Phytoplanktons forms the food of the mussels, and they are filter feeders.

P.viridis in the natural conditions grow to 63 mm in 6 months to 133 mm in 4 years.However , the growth in culture operations have been more than in the natural conditions. In mussel the sexes are separate and the gonads which are located in the body

proliferate into mantle. The male gonad is creamy white in colour while in the female it is pink or reddish. The mussel attains first maturity at 15.5 to 28 mm size.




Inhaled water
Exhaled water
Egestive
sipho
Ingestive
sipho
Plankton
foot
Byssus threads



Technology of mussel culture

A) Seed collection / Availability

The spawning season of the green mussel is between July and September and the spats are found carpeting the inter tidal and submerged rocks.At present they are collected manually and during the peak season an individual would be able to collect 10-12 kg of seed in one hour. The seeds can also be collected using spat collectors such as roof tiles, coir ropes and nylon ropes.

Even though the hatchery technique for commercial mussel spat production has been perfected by Central Marine Fisheries Research Institute, Cochi, there is no commercial hatchery at present in India.As such the culture operations have to depend on the availability of natural seed .

B) Farming models

Three types of farming are practiced for culture of the mussels as follows:

i. Sea Farming

ii) Estuarine farming

iii) Rope culture

i. Sea Farming

Longline culture of mussel is practised in shallow waters of 10 - 15 m depth . This method of culture can withstand the severe monsoon conditions in the west

coast. The longline unit consist of 60 mt long horizontal HPD rope of 20-24 mm thickness anchored at both the ends with 150 Kg concrete blocks and a series of 100 liters capacity barrels as floats fixed at 3 m intervals. Vertical lines of 6 m length seeded with mussel spats are hung at a distance of 75 cm between two floats in the main line. A longline unit of 60x60 mt can accommodate 12 horizontal ropes and 920 - 1000 vertical ropes. The distance between two horizontal lines is 5 mt . At every 20 mt the horizontal lines are connected using additional horizontal lines.

ii) Estuarine farming

Pole culture and stake culture are done in estuaries at a depth of 1.5 to 3 m. The spats of 15 to 25 mm are wrapped around the poles or stakes with cotton mosquito nettings. The spats gets attached to the poles in three or four days and by this time the cotton netting will disintegrate. Periodical thinning is necessary.

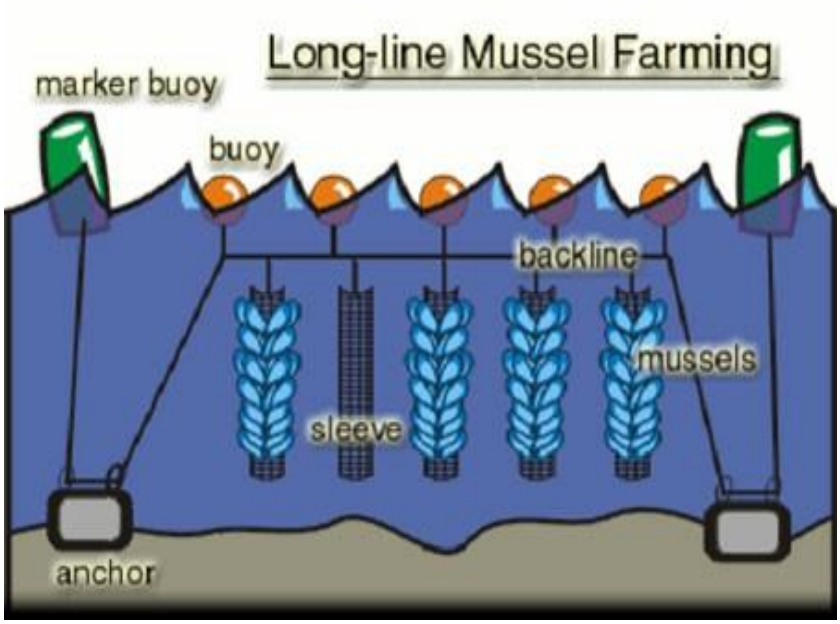
iii) Rope culture

Rope culture of mussel is widely adopted in Northern Kerala. Ropes are suspended from rack made of casuarina and bamboo poles. The average area of rack is 400 sq m and length of the ropes used for seeding ranges from 1-1.25 mt depending on the depth of the water column.Poly propylene ropes wound with coir ropes are used for seeding.

These ropes are hung down from the racks at an interval of 1 feet and nearly 500 - 550 ropes could be suspended from one rack. The seeds collected from wild are being sold in units of one bag and one bag of seed can be used to seed 8-10 ropes. The normal size of the seed ranges from 35-65 mm.

Seed collected has to be seeded on the same day and it is estimated that one person can seed around 60-70 ropes in a day. The culture period in Northern Kerala where the activity is taken up fairly on a large scale starts from November and ends in the middle of May before the rains. Once in a fortnight the ropes are lifted for monitoring the growth and removal of fowling organisms.

The mussel grows to 80-100 mm size with in 6 months of culture period and it is estimated that around 2 lakhs mussels can be harvested from 400 sq mtrs.



4. Processing

Before removing the meat from the mussel it is necessary to carry out depuration which is a process in which the mussels are kept for 18 hours in clean sea water which will purify the mussels of bacterial pollution. The mussels can be processed in different forms like frozen, canned, smoked, dried and marinated. The mussel shell is used as a liming agent in coconut plantations.

The mussel shell gives good quality lime which finds application in many industries.

Financial viability

The following assumptions have been made on the basis of the farming practiced in Kerala for working out the financial viability of the project.

1	Unit size of rack (Area)	400 sq m
2	Culture period	6 months
3	Size of the seed (Spats) at the time of seeding	35-65 mm
4	Size at harvesting	80-100 mm
5	Number of mussel that could be harvested from	400 sq m 2 lakh
6	Production	
7	1st year 70%	

5. Marketing

There is only limited demand for the mussel meat due to lack of awareness among the consumers . However, there is scope for its export to Southeast Asian countries. A marketing tie-up with the processing plants will be useful for marketing of the product.

Marine algal culture (Seaweed culture)

- Seaweeds, which are macroscopic marine algae belong to the primitive non flowering group - Thallophyta.
- They grow submerged and attached to hard substrata such as stones, rocks and coral reefs along the shallow coasts, lagoons, estuaries and
- brackish water habitats of the Andaman - Nicobar and Lakshadweep islands and
- coastal areas of Tamil Nadu, Kerala, Karnataka, Maharashtra, Gujarat, Goa, Orissa and Andhra Pradesh.
- Based on their pigmentation and other morphological characteristics they are categorised into three major groups –
- Chlorophyceae which is popularly known as **green seaweeds**,
- Phaeophyceae or **brown seaweeds** and
- Rhodophyceae or **red seaweeds**.

Uses of seaweeds

- Seaweeds contain more than 60 trace elements in a concentration much higher than in land plants.
- They also contain vitamins, proteins, essential amino acids, iodine, bromine and antibiotics and several bioactive substances.
- They are used as human food, feed for livestock, poultry, fish and prawn and as manure for many plantation crops.
- **Agar is mainly produced from red seaweeds such as**

  Gracilaria edulis,

  Gelidiella acerossa,

  Gracilaria verrucosa and
- ***Carrageenan* from**

  Eucheuma and

Hypnea.

- **Alginic acid and mannitol are manufactured from brown seaweeds such as**

  Sargassum and

  Turbinaria

These Phycocolloids are used in

- food,
- confectionery,
- pharmaceutical,
- biomedical,
- dairy,
- textile,
- paper and
- paint industries as
- gelling,
- stabilizing and
- thickening agents.
- In Japan, Malaysia, China, Philippines and Indonesia,

Green seaweeds such as

- *Ulva*,
- *Enteromorpha*,
- *Caulerpa*,
- *Codium* and
- *Monostroma*;

Brown seaweeds such as

- *Sargassum,*
- *Hydroclathrus,*
- *Laminaria,*
- *Undaria* and
- *Macrocystis* and
- **Red seaweeds such as**
- *Porphyra,*
- *Gracilaria,*
- *Eucheuma,*
- *Hypnea,*
- *Laurencia* and

Acanthophora are **consumed as vegetables, in soups, salads, porridges and pickles.**

- Resource assessment surveys on seaweed conducted by CMFRI, CSMCRI and NIO indicate that the total standing crop along the Indian coastline consists of more than 1,00,000 tonnes
- wet weight per year belonging to 680 species of which 60 species are economically important.
- They comprise 8,000 tonnes of agar-yielding seaweeds, 6,000 tonnes of carrageenan-yielding seaweeds and 16,000 tonnes of algin yielding brown seaweeds.
- Edible and other green seaweeds constitute a bulk of 70,000 tonnes.

Why cultivate seaweeds?

- In India there are about 50 seaweed industry units located in Ahmedabad, Baroda, Cochin, Hyderabad, Madurai and Ramanathapuram.
- They depend only on natural seaweed beds for their raw material.
- A rough estimate indicated that 8,100 tonnes dry weight of agar and algin-yielding seaweeds was utilized by the industry in the year 1995.

- As more and more new industries are coming up every year, exploitation rate exceeds the harvestable biomass.
- The indiscriminate exploitation of these resources from the natural beds leads to shrinking of stock.
- Hence, mariculture of seaweeds all along the Indian coast, estuaries and certain backwaters is the only way to increase the production of phycocolloids and thereby to make the industry commercially attractive.

Advantages of seaweed culture

a) Increases the seaweed production

b) Desirable varieties can be selected and cultivated on a large scale

c) Natural beds can be protected and conserved against over-exploitation

d) Exotic or oriental species of commercial seaweeds such as Eucheuma can be introduced, acclimatised and cultivated in our waters

e) Can support seaweed industry by regular supply of raw materials of same quality and maturity at low cost

How to culture seaweeds?

- The Central Marine Fisheries Research Institute has developed technologies for mariculture of seaweeds especially the agar-yielding red seaweed *Gracilaria edulis* by constant research since 1972.
- Calm and shallow coasts and bays and lagoons with sandy bottom are ideal sites for *G. edulis* culture.
- For optimum growth of this weed a salinity of 28-35 ppt is desirable.
- *G. edulis* can easily be grown to harvestable size within 60 days from small bits of vegetative fronds.

- This is cultivated in 5 x 2 m size net rafts made of coir ropes.
- *G. edulis* stock collected from natural bed are cut into small bits of approximately 5 cm size.
- These bits of about 5 g are inserted between the twists of the rope.
- These floating net rafts are tied to wooden poles that are staked from the sea floor or tied to floats and anchors in places where wooden stakes cannot be fixed.
- Like this about 900 net rafts can be accommodated in a hectare area.
- These rafts are submerged in 30-40 cm water column to avoid desiccation during the ebb tide.

Besides net rafts, *G. edulis* can also be cultivated in long-lines made of thick 10m long coir ropes from which small seeded ropes be suspended at regular intervals.

Seed stock for mariculture can be obtained from Rameshwaram and Kilakkarai of Tamil

Nadu coasts or from the lagoons of Lakshadweep islands.

Once in every fortnight cleaning the rafts is desirable to remove epiphytes and other attached weeds.

- The harvest is made after 60 days by cutting the loosely grown fronds, leaving the base attached to the rope.
- This forms the seed material for the second crop.
- Approximately 30 kg of seaweed per net can be harvested from 10 kg seed stock.
- In this way during the fair weather three or four crops can be cultivated in a year.

The harvested seaweed must be cleaned and dried well before storing. The moisture content in *G. edulis* ranges from 70- 75%.
Hence the dry weed weighs one fourth of the weight of fresh seaweed.
Dried seaweeds are sold to the industry at the rate of Rs.4,000 to 5,000 per tonne. Agar is extracted from the dry seaweed.

- Production of oyster, mussels, cuttlefish, fin-fishes, shrimps, lobsters and seaweeds through aquaculture has increased from just 10.4 million tonnes in 1980 to 22.6 million tonnes in 1990.
- This hike in production is attributed mainly due to 87.2% increase in seaweed.

- China achieved a record production of 2,75,000 tonnes dry weight of *Laminaria* in 1990 from just 62 tonnes in 1952.
- Today Japan stands first in the production of Nori or *Porphyra*, China for *Laminaria*, South Korea for *Undaria edulis* and the Philippines for *Eucheuma*.
- India can take up *Gracilaria* culture and become the largest producer as she is endowed with 8,041 km long coastline and 51.2 km^ of continental shelf area in the form of sheltered bays and lagoons.
- Venturing into mariculture of *G. edulis* along the Kerala coast with the public participation will be a highly profitable and spare time avocation.


2 9:08PM

FISH GENETICS:

Gynogenesis

Gynogenesis is the process of embryonic development with solely the maternal genome and without paternal genetic input, a phenomenon similar to parthenogenesis. Gynogenesis occurs in nature and can also be induced.

Mitotic gynogenesis

Mitotic gynogenesis can be used to create mitotic gynogens (all genes come from the mother), fish that are 100% inbred. The technique that is used to accomplish this with species that have the XY sex-determining system (females are XX and males are XY; virtually all aquacultured species have this system of sex determination) is outlined in Figure.

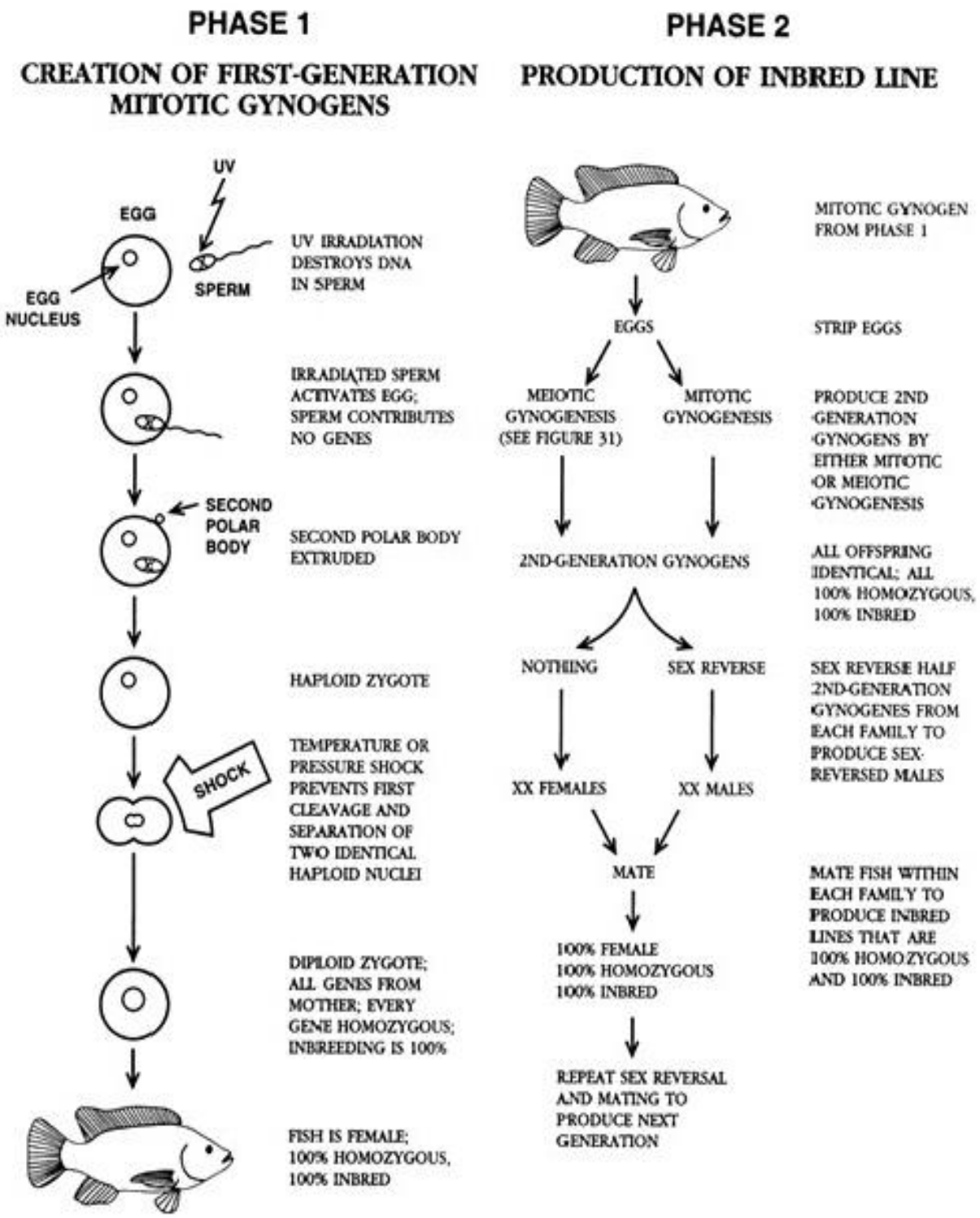
The first step in this breeding programme is the production of first-generation mitotic gynogens. Ultraviolet radiation is used to destroy the DNA (the genes) in sperm. The irradiated sperm are then used to activate eggs. An irradiated sperm cannot fertilize an egg because its genes have been destroyed. The activation causes the egg to undergo the equational division (second meiotic division) and to extrude the second polar body. The egg now contains only a haploid egg nucleus; this produces a haploid zygote (the zygote contains only a single chromosome (homologue) from each chromosome pair, and each chromosome comes from the mother, which is why they are called “gynogens”).

When the haploid zygote undergoes first cleavage, a pressure or temperature shock is used to prevent the haploid zygote nucleus from dividing into two daughter nuclei.If the shock is timed perfectly, the haploid zygote nucleus has replicated its chromosomes so that each daughter nucleus will have a full and identical set of chromosomes, but the haploid zygote nucleus has not divided.By preventing first cleavage, the zygote remains a zygote, but the chromosome number of the zygote has doubled from the haploid state to the normal diploid state, which means that each chromosome occurs as a pair.

Since mitosis (first cleavage is a mitotic cell division) produces two identical sets of chromosomes, each chromosome pair is composed of two identical chromosomes. Consequently, every gene comes from the mother, and every gene is homozygous; the mitotic diploid gynogen is 100% homozygous and 100% inbred. If first-generation mitotic gynogens are to be used in a breeding programme to create inbred lines, a second phase of gynogenesis followed by sex reversal is needed in order to produce the lines of 100% inbred fish, in which all fish within each inbred line are genetically identical.

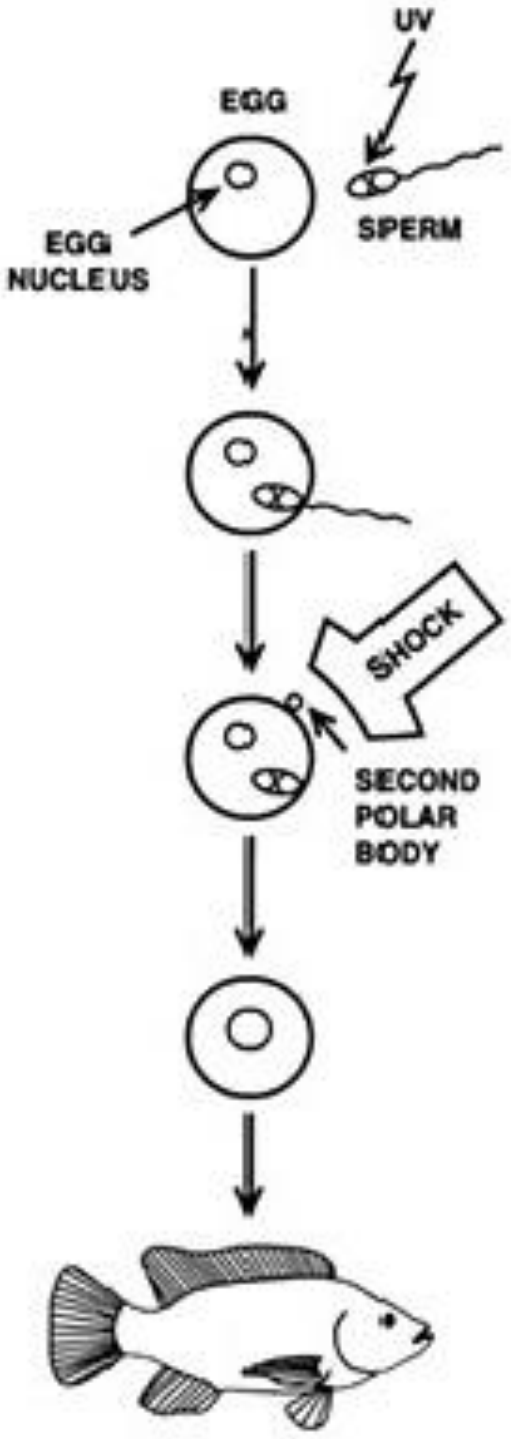
Each first-generation mitotic gynogen will be used to create a unique line of 100% homozygous and 100% inbred fish by utilizing either of two possible types of gynogenesis. When the first-generation mitotic gynogens mature, their eggs are stripped, and either mitotic gynogenesis is repeated to produce second-generation gynogens or meiotic gynogenesis is used to create second generation gynogens.

Half of the second-generation gynogens from each family are sex-reversed with anabolic androgens (steroid hormones) to produce XX sex-reversed males. The sex-reversed males are genetic females but phenotypic males. The fish that are not treated with hormones are raised normally. Within each family, the sex-reversed males and their sisters are genetically identical (genetically, they are all identical sisters); when they mate, they produce an inbred line of genetically identical fish that is 100% female, 100% homozygous, and 100% inbred. Sex-reversed males must be created every generation, because it is the only way males can be produced, and it is the only way each inbred line can be perpetuated without additional chromosomal manipulation.


PHASE 1
CREATION OF FIRST-GENERATION MITOTIC GYNOGENS
PHASE 2
PRODUCTION OF INBRED LINE
UV
EGG
EGG NUCLEUS
SPERM
UV IRRADIATION DESTROYS DNA IN SPERM
IRRADIATED SPERM ACTIVATES EGG; SPERM CONTRIBUTES NO GENES
SECOND POLAR BODY
SECOND POLAR BODY EXTRUDED
HAPLOID ZYGOTE
SHOCK
TEMPERATURE OR PRESSURE SHOCK PREVENTS FIRST CLEAVAGE AND SEPARATION OF TWO IDENTICAL HAPLOID NUCLEI
DIPLOID ZYGOTE; ALL GENES FROM MOTHER; EVERY GENE HOMOZYGOUS; INBREEDING IS 100%
FISH IS FEMALE; 100% HOMOZYGOUS, 100% INBRED
MITOTIC GYNOGEN FROM PHASE 1
EGGS
STRIP EGGS
MEIOTIC GYNOGENESIS (SEE FIGURE 31)
MITOTIC GYNOGENESIS
PRODUCE 2ND GENERATION GYNOGENS BY EITHER MITOTIC OR MEIOTIC GYNOGENESIS
2ND-GENERATION GYNOGENS
ALL OFFSPRING IDENTICAL; ALL 100% HOMOZYGOUS, 100% INBRED
NOTHING
SEX REVERSE
SEX REVERSE HALF 2ND-GENERATION GYNOGENES FROM EACH FAMILY TO PRODUCE SEX-REVERSED MALES
XX FEMALES
XX MALES
MATE
MATE FISH WITHIN EACH FAMILY TO PRODUCE INBRED LINES THAT ARE 100% HOMOZYGOUS AND 100% INBRED
100% FEMALE
100% HOMOZYGOUS
100% INBRED
REPEAT SEX REVERSAL AND MATING TO PRODUCE NEXT GENERATION

Meiotic gynogenesis

Gynogenesis can be used to create another type of inbred fish-meiotic gynogens. This type of chromosomal manipulation is easier than mitotic gynogenesis, and meiotic gynogens have a higher survival rate than mitotic gynogens because they have less inbreeding.Meiotic gynogenesis is less useful in producing inbred lines because it is difficult to accurately predict the exact amount of inbreeding produced, and the inbreeding produced each generation is quite variable.Since regular systems of inbreeding are most useful when they produce reliable and predictable amounts of inbreeding, meiotic gynogenesis is less useful than mitotic gynogenesis for producing inbred lines. However, one to three generations of meiotic gynogenesis can be used to produce highly inbred fish.

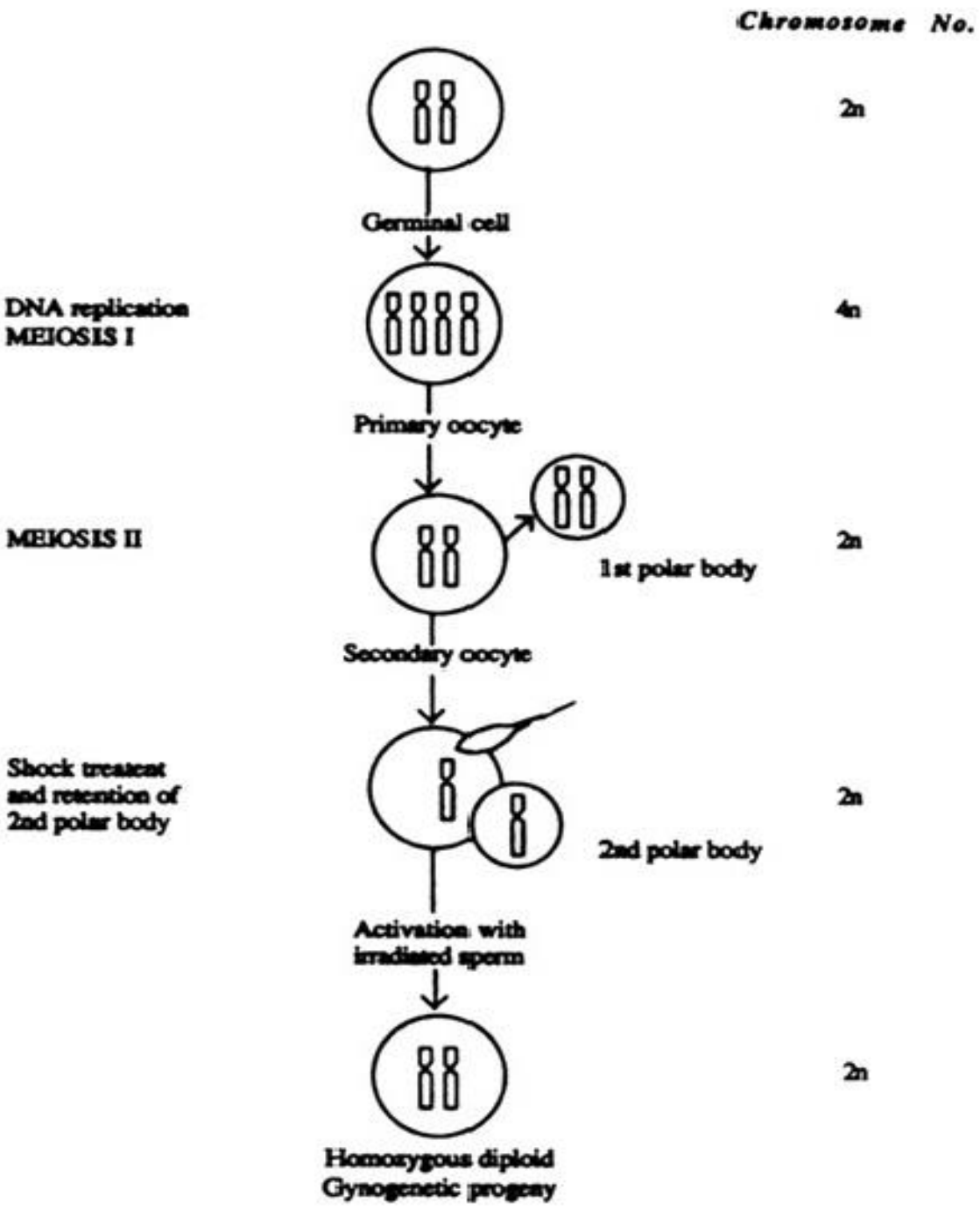


UV IRRADIATION DESTROYS DNA IN SPERM

IRRADIATED SPERM ACTIVATES EGG; SPERM CONTRIBUTES NO GENES

TEMPERATURE OR PRESSURE SHOCK PREVENTS EXTRUSION OF SECOND POLAR BODY

SECOND POLAR BODY NUCLEUS FUSES WITH EGG NUCLEUS TO FORM DIPLOID ZYGOTE; ALL GENES FROM MOTHER


Chromosome No.
2n
Germinal cell
DNA replication
MEIOSIS I
4n
Primary oocyte
MEIOSIS II
2n
1st polar body
Secondary oocyte
Shock treatent
and retention of
2nd polar body
2n
2nd polar body
Activation with
irradiated sperm
2n
Homozygous diploid
Gynogenetic progeny

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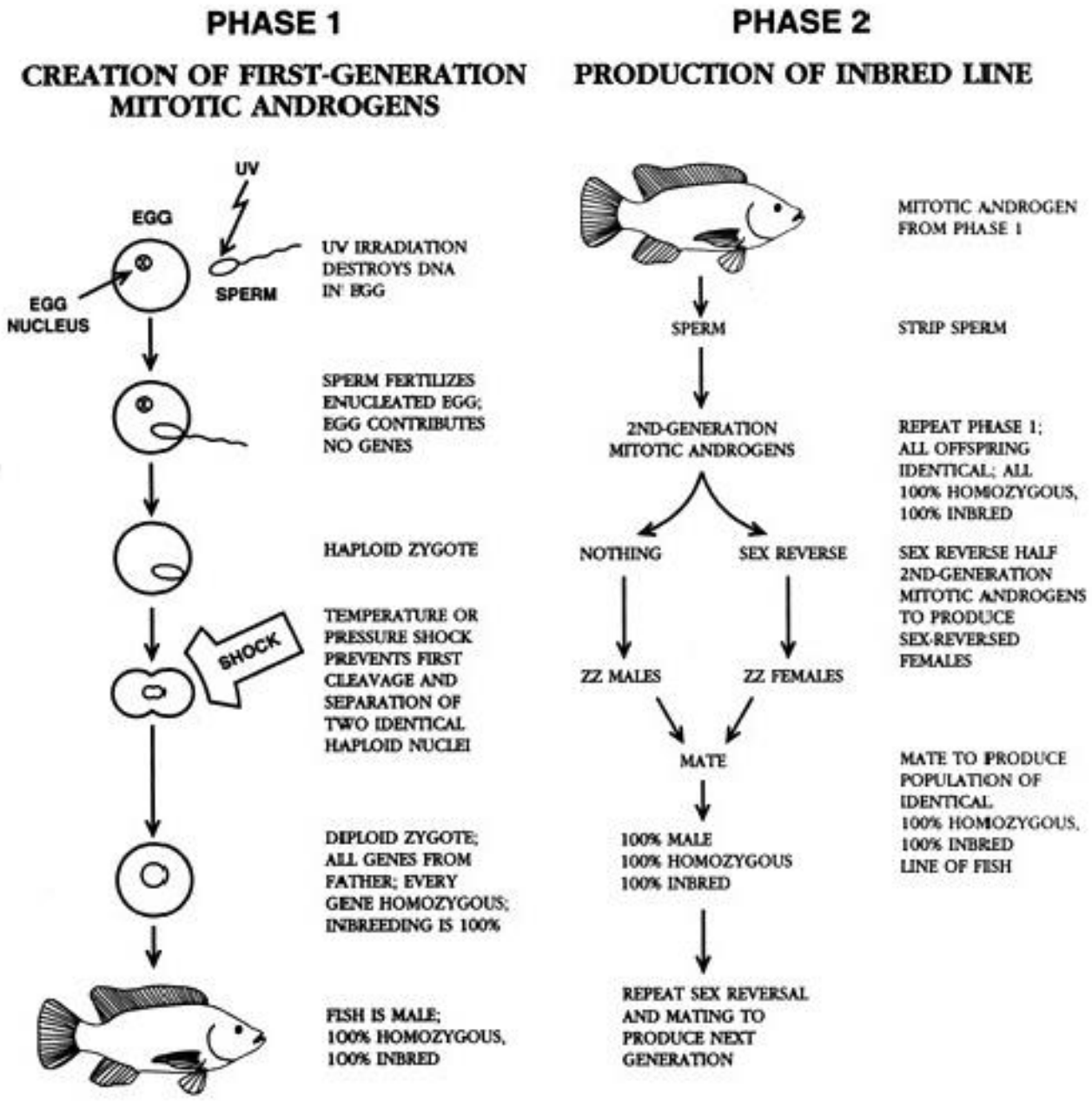
Mitotic androgenesis

Mitotic androgenesis can be used to produce mitotic androgens (all genes come from the father), fish that are 100% inbred. The technique that is used to produce mitotic androgens for species with the WZ sex-determining system is outlined in Figure



Phase 2 of this breeding programme uses a second round of mitotic androgenesis, and half the offspring from each family are sex-reversed with anabolic estrogens to produce sex-reversed ZZ females. These females are genetic males but phenotypic females. Within each family, the sex-reversed females are mated to their genetically identical brothers to produce each inbred line; fish in each inbred line are genetically identical, 100% homozygous, and 100% inbred and all are males. As was the case with mitotic gynogens, each line is genetically unique.

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Polyploidy

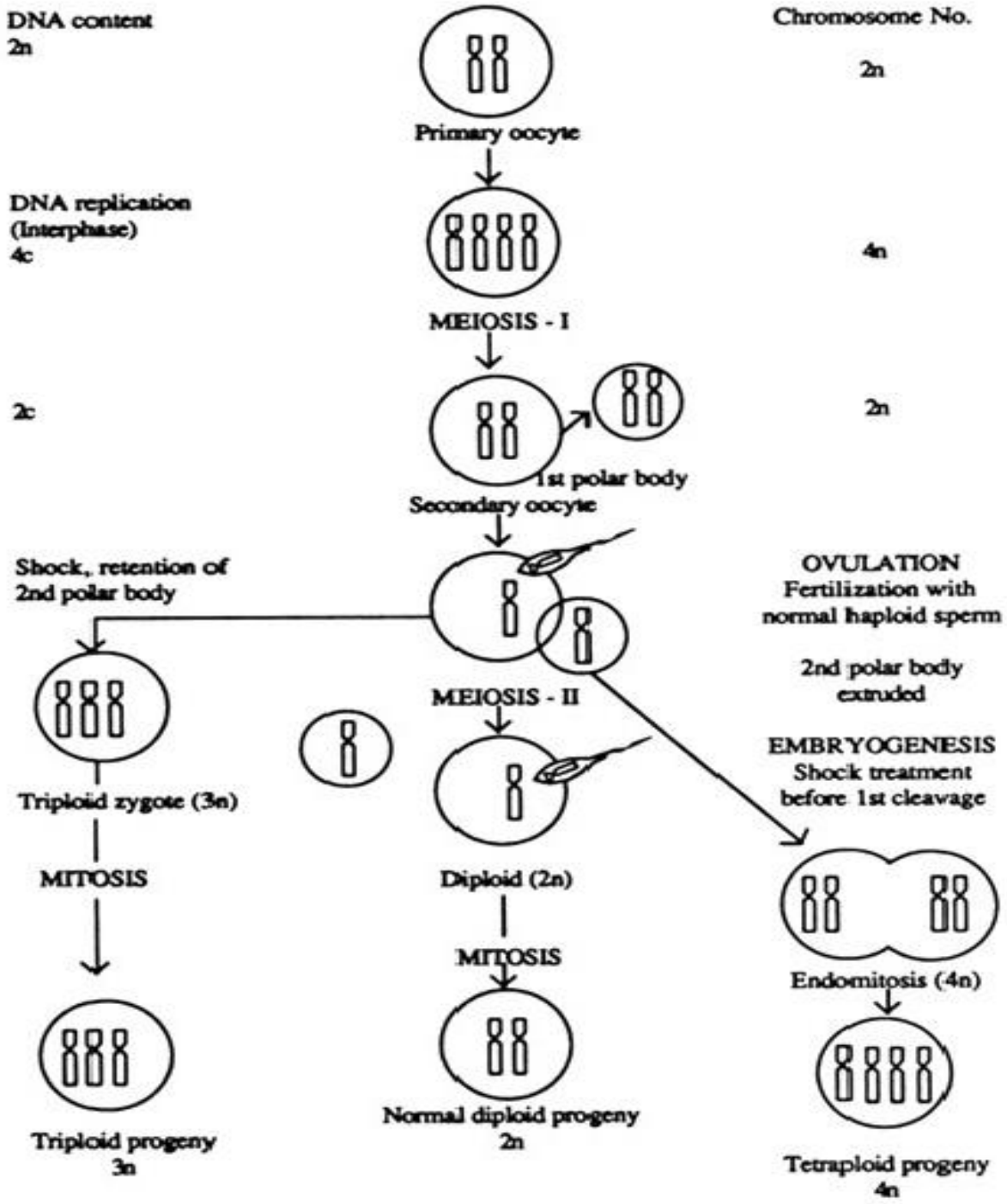
An increase in the level of ploidy of an individual by the addition of one or more set(s) of chromosomes refers to polyploidy resulting usually either in triploidy or tetraploidy.Sometimes it may also result in penta or hexaploidy.

Natural polyploidy (triploidy/tetraploidy)

Polyploidy has been observed to occur in nature in some species of fish like the common carp (*Cyprinus carpio*) and trout mainly due to chromosomal translocation and when two distantly related fish species are crossed.The crosses between grass carp and bighead carp had produced triploid hybrids (Marian and Krasznai, 1978).However, none of the interspecific nor intergeneric hybrid crosses among Indian carps have been reported to produce such allotriploids.

Artificial induction of polyploidy in Indian major carps

Some preliminary attempts have been made to induce artificial induction of triploidy and tetraploidy in Indian major carps with varied degrees of success


DNA content
2n
Chromosome No.
2n
Primary oocyte
DNA replication
(Interphase)
4c
4n
MEIOSIS - I
2c
2n
1st polar body
Secondary oocyte
Shock, retention of
2nd polar body
OVULATION
Fertilization with
normal haploid sperm
2nd polar body
extruded
MEIOSIS - II
EMBRYOGENESIS
Shock treatment
before 1st cleavage
Triploid zygote (3n)
MITOSIS
Diploid (2n)
MITOSIS
Endomitosis (4n)
Triploid progeny
3n
Normal diploid progeny
2n
Tetraploid progeny
4n

Conclusion:

Chromosomal manipulation can be used to quickly produce highly inbred lines of fish.One generation of mitotic gynogenesis or mitotic androgenesis will produce fish that are 100% homozygous and 100% inbred. A second generation of chromosome set manipulation is needed to produce 100% inbred fish that are capable of reproducing.

One generation of meiotic gynogenesis will produce fish that have large, but unknown, levels of inbreeding; the amount of inbreeding produced by meiotic gynogenesis is variable and depends on crossing over frequencies.The use of chromosomal manipulation to produce inbred lines should be done only by scientists at large agribusinesses or at research stations.

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Artificial insemination

Artificial insemination (the collection of spermatozoa and ova and their mixing together in various media that keep spermatozoa motile) is carried out in only a few species (mostly freshwater), such as salmonids, cyprinids and acipenserids. Traditionally, fresh water (or sea water for marine species) is used as the medium in which the male and female gametes are mixed. However, fresh water is not a very favourable medium because hypotonic shock causes the sperm structure to deteriorate in several minutes and the egg is activated quickly. These problems can be avoided by using as media various saline solutions of different composition, depending on the species (125 mM NaCl pH 9 for salmonids; 50 raM Nacl pH 8 for cyprinids). These media prevent sperm deterioration, prolong slightly the duration of motility, and prevent or defer the cortical reaction. These solutions also prevent the yolk of crushed eggs from precipitating when it comes into contact with the water, limit motility and block the micropyle. Fish farmers are beginning to use these media, so significantly increasing the fertilization rate while reducing the number of spermatozoa used for insemination. The length of gamete survival is an important factor to consider in carrying out artificial reproduction. Gamete survival *in vivo* (after the release of sperm and oocytes from cysts and follicles) varies with the species. Sperm fertilizing ability decreases during the spawning period in sea bass and trout but not in carp. Ovum survival in the general or ovarian cavity is from one to several weeks in salmonids, several hours in carp at 20°C and only 30 min in Chinese carp. *In vitro* survival is from one to several weeks for sperm (under oxygen and with antibiotics added) and several hours for ova (2–4 h in carp and 12–24 h in trout). The spermatozoa of several species of teleosts have been stored deep frozen, but the quality of the sperm is not as good and more spermatozoa per ovum have to be used to obtain the same percentage of fertilization as with non-frozen sperm.

INDUCED BREEDING AND LARVAL REARING of Clarias macrocephalus

1. INTRODUCTION

Walking catfish in English, or “pla duk” in Thai, is a generic name for a number of species belonging to the family Clarridae. Five are encountered in Thailand, two of which are popular sources of animal protein, Clarias batrachus and Clarias macrocephalus, locally known as “pla duk dan” and “pla duk oui”, respectively. C. batrachus fry is easily obtained from the spawning pond. Unfortunately, C. macrocephalus do not readily re roduce themselves in captivity. However, it can be induced to breed if injected with extracts of fish pituitary glands containing gonadotropin sex hormones.

Thai consumers have a preference for C. macrocephalus but, because of bottlenecks in fry availability and slow growth, its culture is still limited in comparison to C. batrachus.

Our biologists have worked for 20 years to develop artificial breeding methods. This attempt was first successful for Pangasius sutchi and Chinese Carps in 1965. Further studies were continued. Up to now, induced spawning is a rutine work for our aquaculturi ts.

The purpose of this review is to summarize the induced breeding and larval rearing of C. macrocephalus practices in Thailand.

2. Characteristics and Biology

The species is closely related to C. batrachus from which it can be distinguished by the wide occipital process (Fig. 1). Body is elongate with head broadly depressed, four pairs of well developed barbells, and small eyes. Dorsal and anal fins are long without spine. Pectoral fin has a pungent spine with serrated on its inner edge. Caudal fin is not confluent with dorsal or anal fin (Fig. 2). Body color is dark brown with purplish tint and about tent transverse rows of small white spots on the side.

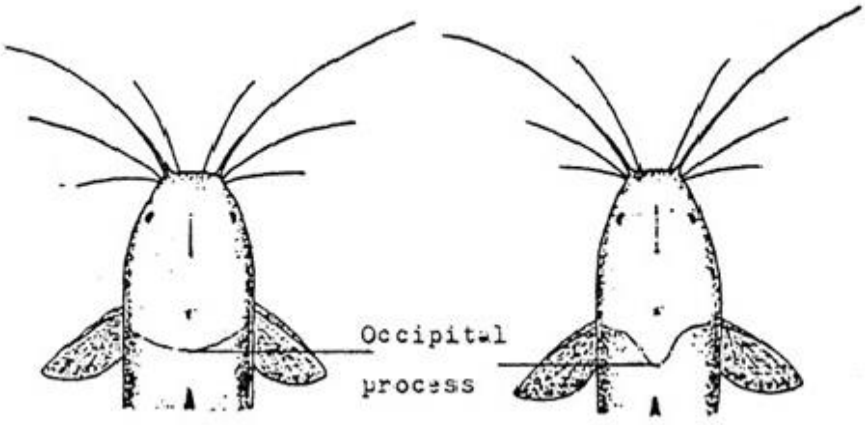


Fig. 1. Occipital process of Clarias macrocephalus (left) and Clarias batrachus (right).

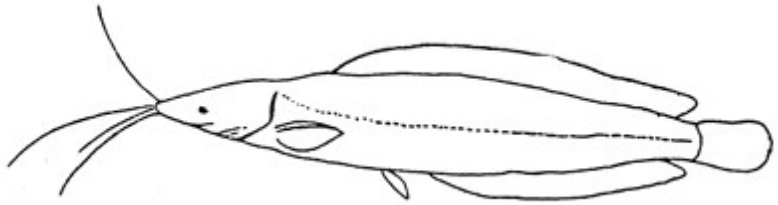


Fig. 2. Clarias macrocephalus Günther

The species is distinguished by their ability to survive in a wide range of water conditions. It requires a relatively small area for culture and can be stocked more densely than many other species. They can live out of water for several hours or in waters of low oxygen content as they have accessory organs that enable them to breathe atmospheric air. Its range of distribution includes the areas from Indochina penninsular. Thailand and the Philippines.

The spawning season is between May and October. The female makes a small round hollow nest with grassy bottom about 30 cm in diameter and 5 – 8 cm deep in shallow water. The eggs are deposited in the nest and attached to the roots of aquatic vegetation in the nest. The male will take charge of these eggs until they are hatched out. The egg can be hatched out within 20 hours at temperature of 25 – 30°C. A female weighing 300 – 800 gm can produce between 5,000 – 10,000 eggs. The natural diet is wide-ran ing, it includes worms, insects, shrimps and decayed matter.

3. Selection of Spawners

The essentials in fish induced spawning are fully ripe mature brooders both female and male. Brood fish should be carefully tended for two to three months before induced spawning operations are carried out. Males and females should be segregrated and stocked in separate ponds. Selection of spawners is one of the most important stages in induced spawning operations. It is necessary to know how to select healthy males and females in order to obtain maximum production of fry.

Determination of ripeness is an art and requires experience. To be good brooders the firsh must be more than one year old or 150 gm. Sex can be distinguished by the shape of the genital papilla (Fig. 3). The male genital papilla is pointed. The female papilla is oval shape. The following characteristics can be used as guidelines to ascertain that the female is ready for induced spawning operations. It has a bulging abodomen. It is clastic and soft to the touch. The cloaca is reddish and prominent, and the contour of this ovary can be seem on both sides of the abdomen.

4. Obtaining the Pituitary Gland

Pituitary gland contains hormone namely gonadotropin which stimulate the production of sex steroids in the gonad which responsible for the maturation of gametes. Gonadotropin is composed of follicle stimulating hormone (FSH) and luteinizing hormone (LH) which are responsible for egg development and egg ovulation respectively.

Pituitary gland of common carp, Chinese carp, Indian carp, and Pangasius sutchi can be used for induced spawning. The fish from which the hyopphysis is to be collected is weighed and placed on a shopping board. The skull is cut open with a knife (Fig. 4). After removing a piece of the skull, fatty tissue and blood are wiped off with a cotton pad. The pituitary gland can be seen after the mid-brain has been folded back by using forceps.

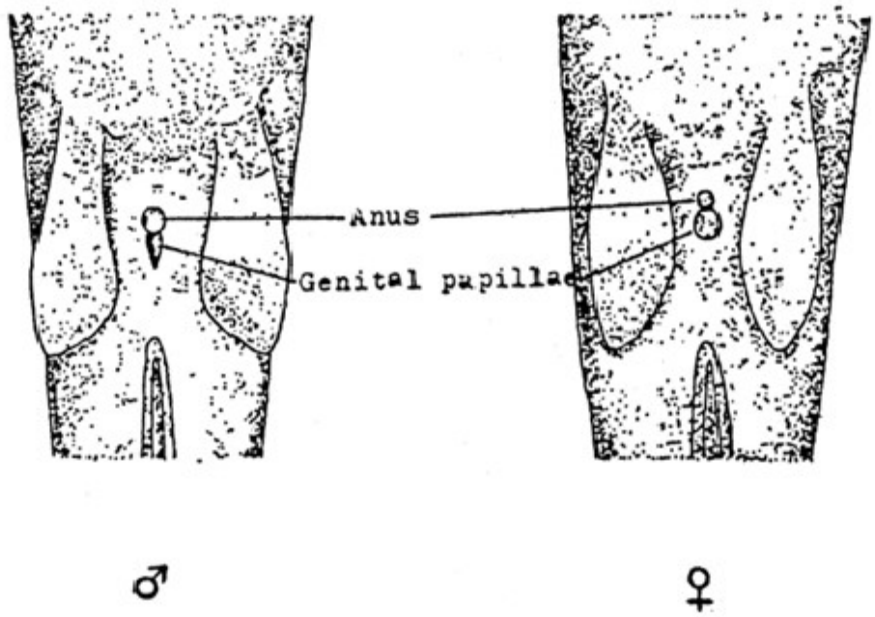


Fig. 3. Genital papillae of the male and female Clarias macrocephalus

5. Amount of Pituitary Gland Solution Injected

One concentration or one dose commonly used can be expressed as follow:

$$\text{one dose} = \frac{\text{weight of donor fish}}{\text{equivalent weight of recipient fish}}$$

The gland is ground in the homoginizer: distilled water is added and the gland is again ground. A syringe is used to take up the solution for injection (Fig. 5). The female can be injected with aypophysis of Chinese carp in a dosage of 1.0 for the first injection and 2.0 for the second injection with the time interval of 6 hours. Aliquots of isotomic saline solution or distilled water is added depending on the weight of recipient, about 0.5 ml for fish weigh less than 1.0 kg of body weight and 1.0 kg body weight and 1.0 ml for fish weigh 1 – 3 kg.

The intramuscular injection is given in the area between the base of the dorsal fin and lateral line (Fig. 6).

6. Artificial Insemination

Ovulation occurs about ten hours after the second injections. The water on the female's body should be wiped off with a towel. As the abdomen is being pressed, the stripped eggs should be collected in a dry plastic container (Fig. 7). At the same time, the milt is made to drip on the eggs by grinding the testis with fingers and pouring the water through the fine mesh cloth. Eggs and sperm are mixed and stirred gently with a feather. Next, a little clean water is added and gently mixed again. After one to two minutes, water is added two or three times to cleanse the fertilized eggs. The fertilized eggs are transferred to the hatching hapa (Fig. 8). Most of the fertilized eggs hatch out within 24 hours. Figure 9 shows the location of testis.

7. Fry Mursing

After yolk resorption, usually within 2 days, the larvae are transferred from hapa to the nursery fiber glass tank. The fry develop feeding behaviour at about the same time their yolk was absorbed. The food to be given for the first 3 weeks is live moina. Usually 3 weeks old fry with the size of 2 – 3 cm are distributed to the fish farmers.

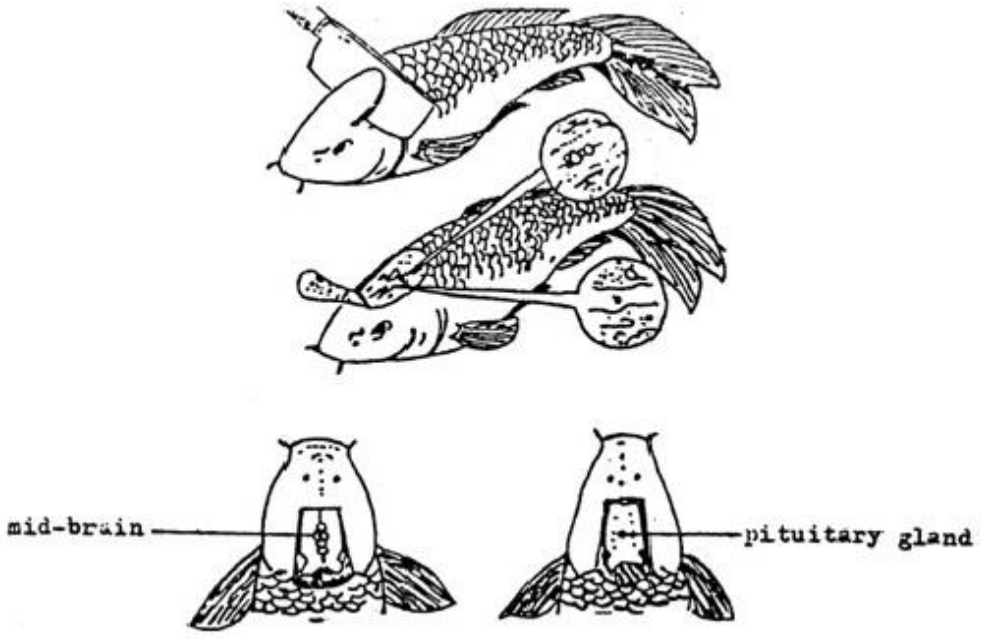


Fig. 4. Extraction method for removing the pituitary gland, showing transverse cut, mid-brain and pituitary gland location.

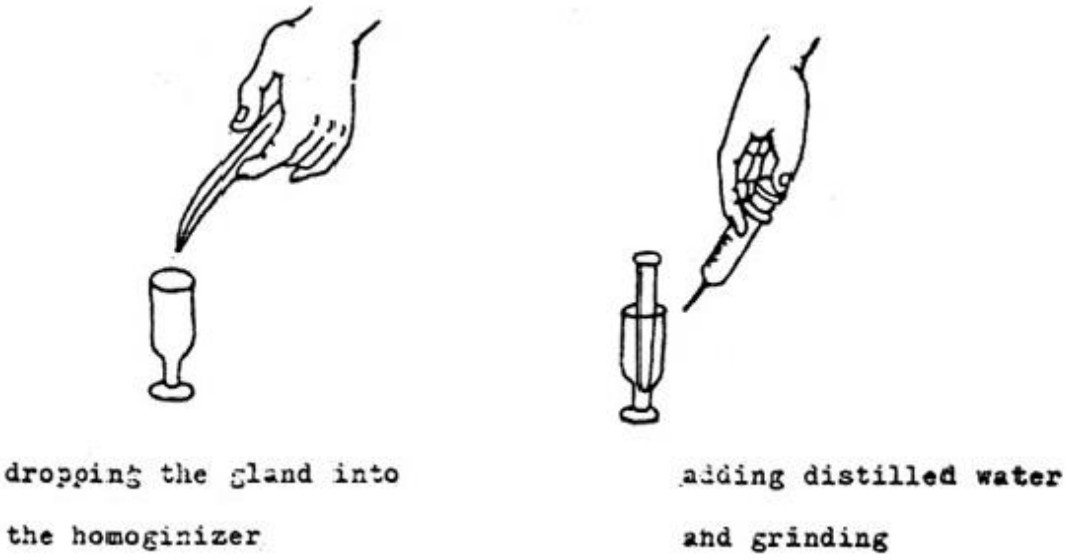


Fig. 5. Preparation of the pituitary gland solution

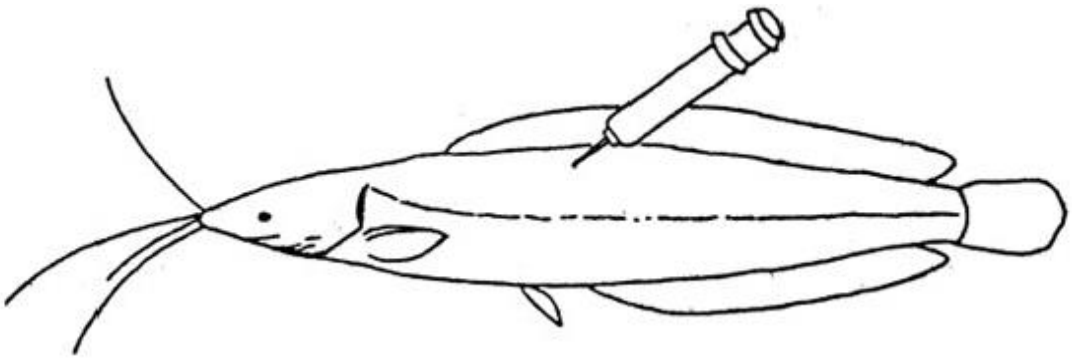


Fig. 6. Location for intramuscular injection.

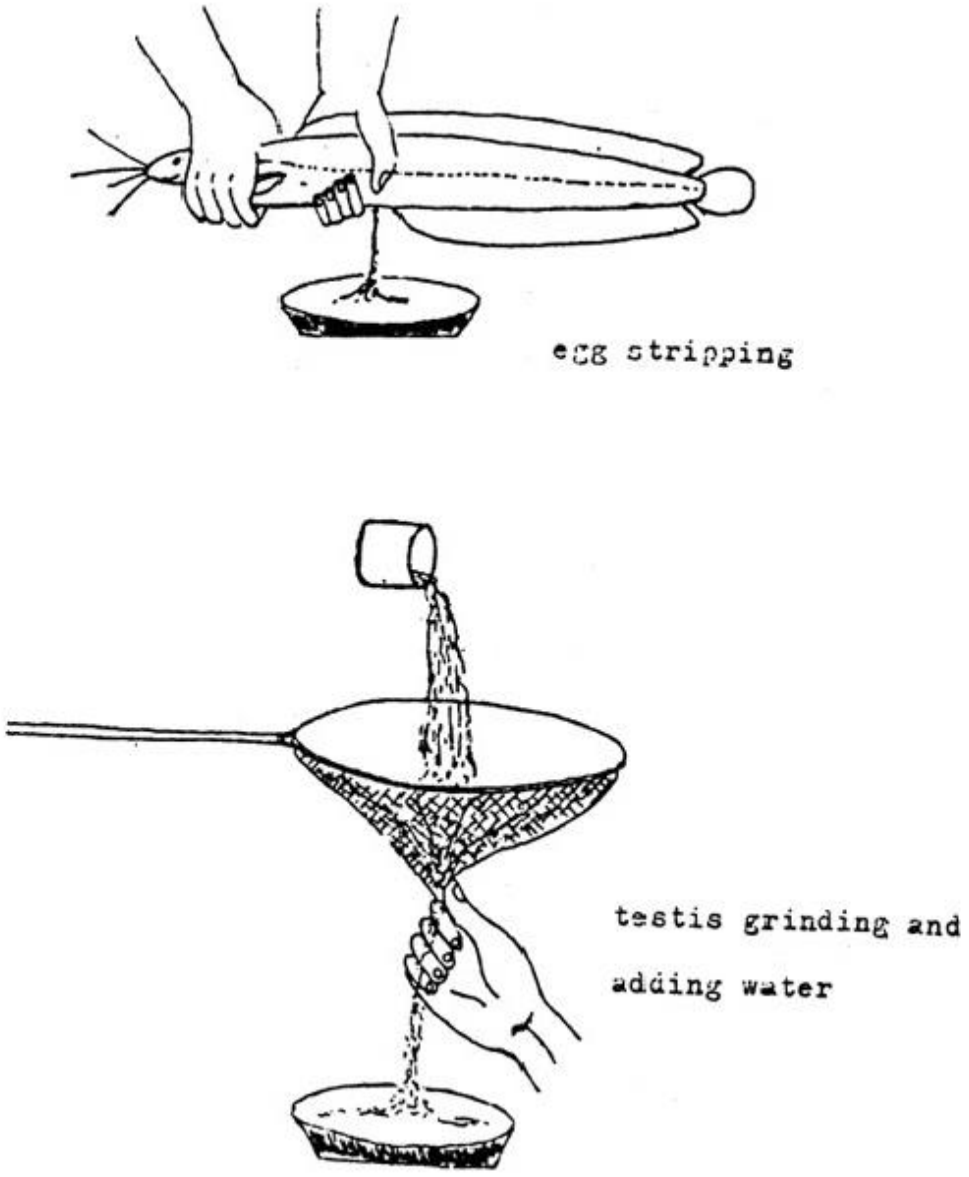


Fig. 7. Artificial insemination.

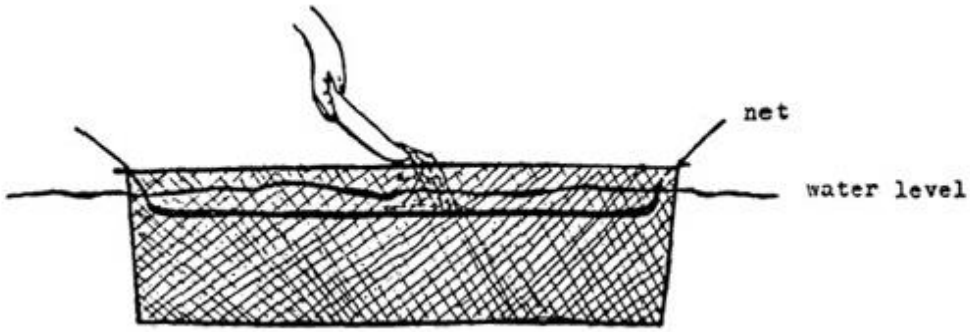


Fig. 9. Transferring fertilized eggs to incubator hapa.

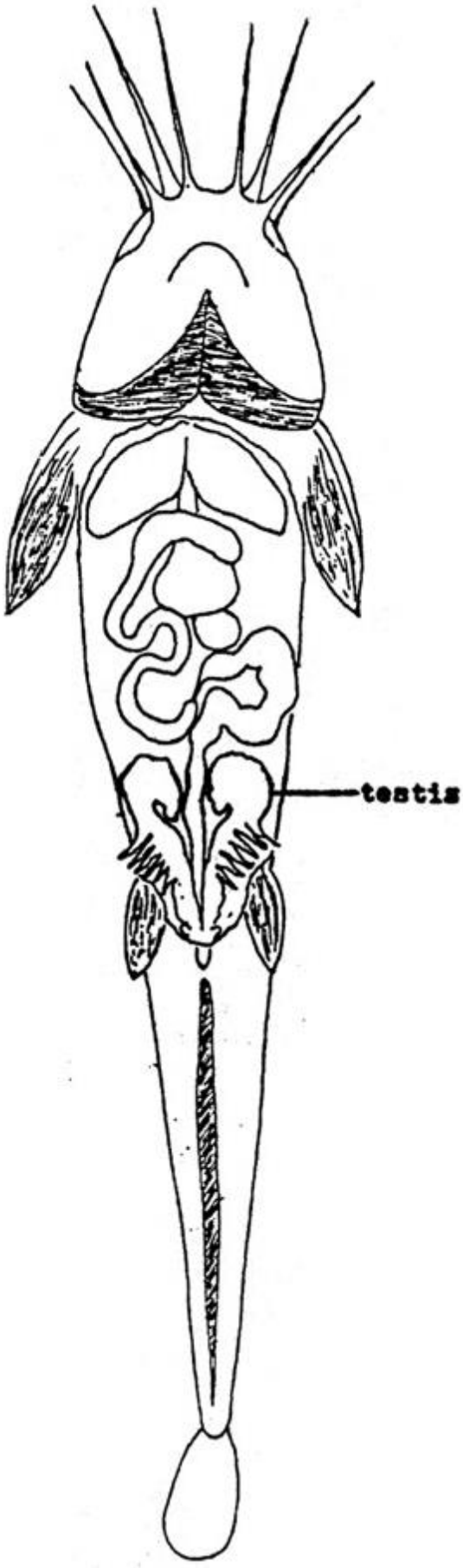

testis

Fig. 8. Location of testis with the digestive track folded up.

8. Egg Development

The stages of egg development are given in Fig. 10.

Fig. 10. Egg development of Clarias macroccphalus, 70 X

Eyestalk ablation

The process of unilateral eyestalk ablation is used in almost every marine shrimp maturation/reproduction facility in the world, both research and commercial, to stimulate female shrimp to develop mature ovaries and spawn. This method of inducing females to develop mature ovaries is used for two reasons:

1. most captive conditions cause inhibitions in females which keep them from developing mature ovaries in captivity
2. even in conditions where a given species will develop ovaries and spawn in captivity, use of eyestalk ablation increases total egg production and increases the percentage of females in a given population which will participate in reproduction

Hormonal effects of eyestalk ablation

The most commonly accepted theory is that a gonad inhibitory hormone (GIH) is produced in the neurosecretory complexes in the eyestalk. This hormone apparently occurs in nature in the non- breeding season and is absent or present only in low levels during the breeding season. By inference, then, the reluctance of most penaeids to routinely develop mature ovaries in captivity is a function of elevated levels of GIH, and eyestalk ablation lowers the high hemolymph titer of GIH. The effect of eyestalk removal is not on a single hormone such as GIH, but rather effects numerous physiological processes (Bray & Lawrence, 1992).

Indirect effects of eyestalk ablation

Considering that eyestalk ablation affects the hormone balance for numerous physiological processes in addition to stimulation of gonadal hypertrophy, what are the practical effects of this operation, and at what cost do we achieve induced ovarian

The following observations have been made concerning use of eyestalk ablation in captive reproduction, and may be related to either captive conditions, eyestalk ablation, or both:

- Captive spawn size (number of eggs per spawn) is smaller than in wild-matured females, regardless of whether eyestalk ablation is used.
- Eyestalk ablation increases total egg production in captivity by producing more frequent spawnings, but not larger spawns.
- There is not a strong trend toward diminishing spawn size over time.
- Molt cycle duration is shorter in eyestalk-ablated females than intact females.
- Higher mortality of eyestalk ablated females is often, but not always, reported.
- Eyestalk ablation has been suggested to deteriorate female condition.
- Eyestalk ablation in some instances has been observed to produce lower hatch rate of eggs than unablated females.
- Hatch rate has been observed to decline over time under captive conditions.
- Ovarian color in captive females, especially in eyestalk ablated females, is often rather different than wild-matured (Bray & Lawrence, 1992).

There is strong circumstantial evidence that part of the problems seen with captive reproduction are related to a simple inability of current diets to supply required nutrients as rapidly as required for the gonadal hypertrophy stimulated by eyestalk ablation. In nature, an organism would not be anticipated to develop eggs, constituting some 10% of female body weight, unless nutrients are available for first, metabolism, second, growth, and third, reproduction. Eyestalk ablation accelerates the production of ova, regardless of whether the proper types and balance of nutrients are available, and regardless of whether those ova are even capable of fertilization. Dietary factors clearly have been shown to influence percentage hatch and percentage of females spawning (Bray & Lawrence, 1992).

Eyestalk ablation techniques Shrimp should be ablated only when hard-shelled, never when in post-molt (newly molted or seft-shelled) or premolt stages. Because molting and reproduction, especially in females, are both energy demanding processes, they appear to be antagonistic in terms of biological programing.

Unilateral eyestalk ablation is accomplished in the following ways:

1) Simple pinching of the eyestalk, usually performed half to two-thirds down the eyestalk. This method may leave an open wound.

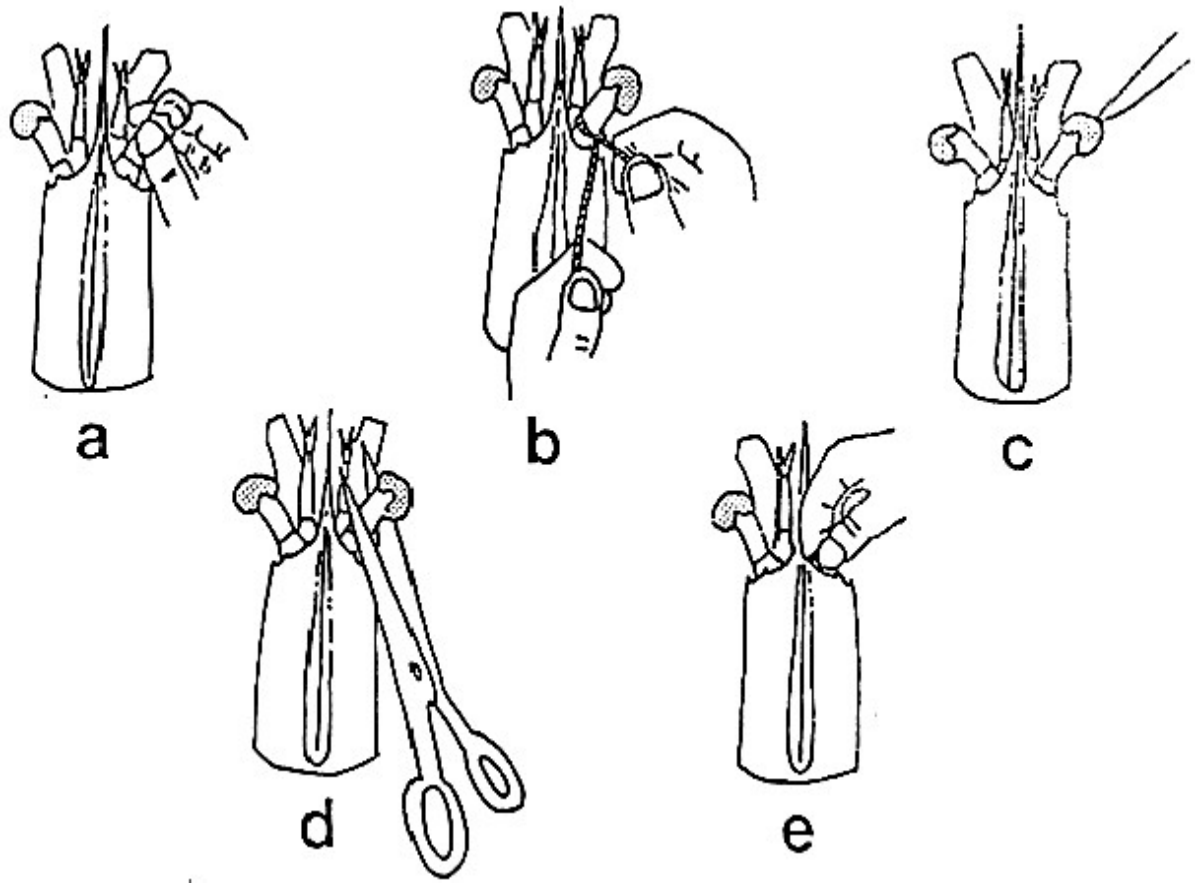
2) Slitting one eye with a razor blade, then crushing eyestalk, with thumb and index fingernail, beginning one-half to two-thirds down the eyestalk and moving distally until the contents of eyes have been removed. This method, sometimes called enucleation, leaves behind the transparent exoskeleton so that clotting of hemolymph, and closure of the wound, may occur more rapidly.

3) Cauterizing through the eyestalk with either an electrocautery device or an instrument such as a red-hot wire or forceps. If correctly performed, this method closes the wound completely and

allows scar tissue to form more readily. A variation of this technique is to use scissors or sharp blade to sever the eyestalk, and then to cauterize the wound.

4) Ligation by tying off the eyestalk tightly with surgical or other thread. This method also has the advantage of immediate wound closure (Bray & Lawrence, 1992).

Latency period: eyestalk ablation to ovarian development
Once females have been subjected to eyestalk ablation, complete ovarian development often ensues within as little as 3 to 10 days, assuming the animals were removed from a breeding or ready-to-breed population, of adequate size for reproduction, and not subjected to too much transfer stress. If the animals have been removed from non-conducive environmental conditions (e.g, cold, non-breeding season temperatures, or hypersaline conditions), a longer than normal latency period between eyestalk ablation and ovarian development can be anticipated, probably due to seasonal hormonal cycling. Duration of the latency period between eyestalk ablation and maturation of ovaries is determined by the readiness of the population at the time of eyestalk ablation (Bray & Lawrence, 1992).



Methods of eyestalk removal in Penaeids:

a) eyeball incision and squeezing;

b) ligation or tying

c) electrocautery or using a silver nitrate bar;

d) cutting;

e) pinching-crushing.

CRYOPRESERVATION

ABSTRACT

Cryopreservation is a long-term storage technique to preserve the biological material without deterioration for extended period of time at least several thousands of years. The ability to preserve and store both maternal and paternal gametes provides a reliable source of fish genetic material for scientific and aquaculture purposes as well as for conservation of biodiversity. Successful cryopreservation of fish sperm have been achieved for more than 200 fish species and many fish species have been adequated for the purpose of cryobanking. Cryopreservation of fish embryo is not viable, mainly because of the same limitations as in fish oocytes, i.e., high chilling sensitivity and low membrane permeability. However, cryopreservation of isolated embryonic cells is another option for preserving both maternal and paternal genome. In this paper, an overview of the current state of aquatic species is followed by a discussion on the sperm, embryos, oocytes and embryonic cells - blastomeres.

Key words: cryopreservation, sperm, embryo, oocyte, blastomere

INTRODUCTION

Cryopreservation is a long-term storage technique with very low temperatures to preserve the structurally intact living cells and tissues for extended period of time at a relatively low cost. Cryopreservation is to preserve and store the viable biological samples in a frozen state over extended periods of time. A very important part research in cryopreservation is to reveal the underlying physical and biological responses of the cell and cause of cryoinjury, especially those associated with the phase change of water in extracellular and intracellular environments (Mazur 1984). From the original slow-cooling study, another cryopreservation approach has moved to easier and more efficient technique-vitrification, Cryoprotective agents has to gain access to all the parts of the system. Cryopreservation considers the effects of freezing and thawing. Therefore, the diffusion and osmosis processes have important effects during the introduction of cryoprotective agents, the addition or removal of cryoprotectants, the cooling process, and during thawing. These phenomena are amenable to the experimental design and analysis. Thus, reliable methods can be developed for preserving a very wide range of cells and some tissues. These methods have found widespread applications in biology, biomedical technology and conservation.

Germplasm cryopreservation includes storage of the sperm, eggs and embryos and contributes directly to animal breeding programmes. Germplasm cryopreservation also assist the *ex situ* conservation for preserving the genomes of threatened and endangered species. The establishment of germplasm banks using cryopreservation can contribute to conservation and extant populations in the future. Since the first successful cryopreservation of bull semen (Polge et al. 1949), cryopreserved bull semen has been used to propagate the rare and endangered species using assisted reproduction techniques. Every year, more than 25 million cows are artificially inseminated with frozen-thawed bull semen (Foote 1975) and many bovine calves have been produced using the transfer of cryopreserved embryos into cow (Mapletoft and Hasler 2005). Tissues, cultured cell lines, DNA and serum samples could be frozen and store in cryogene bank. For example, mice and sheep have been generated from frozen-thawed pieces of ovary that have been replaced in a female and stimulated to ovulation. (Gosden et al. 1994; Candy et al. 2000; Sapundzhiev 2008). The principle of testicular cell freezing and transplantation has been demonstrated and is currently used for human male infertility (Clouthier

et al. 1996). Significant efforts are being made on non-mammalian species using cryobiology techniques. In fish aquaculture, the successful cryopreservation of gametes and embryos could offer new commercial possibilities, allowing the unlimited production of fry and potentially healthier and better conditioned fish as required. Cryopreservation of reproductive products of many aquatic species has been successfully achieved. Cryopreservation of aquatic sperm is relatively common in the breeding and management of fish species, including salmonid, cyprinids, silurids, and Acipenseridae (família) is well documented (Magyary et al. 1996; Tsvetkova et al. 1996). However, cryopreservation of embryos and oocytes of aquatic species have not been successful, except for eastern oyster eggs (*Crassostrea virginica*) (Tervit et al. 2005), larvae of eastern oyster (Paniagua-Chavez and Tiersch 2001) and larvae of the sea urchin (Adams et al. 2006).

Cryopreservation technology applied to the preservation of fish gametes in aquaculture plays an important role in seed production, genetic management of broodstock and conservation of aquatic resources. Fish germplasm also plays a significant role in human genomic studies because its relatively small size of the genome makes it easier for sequencing and ideal models for studying the human disease. This would help in identifying the roles for human genes from fish mutations and also in fish models for genes identified by human disease (Brownlie et al. 1998; Barbazuk et al. 2000). Aquatic species preservation would assist the development, protection and distribution of research lines and would offer benefits for restoration of endangered species.

Sperm

In 1949, Polge et al. (1949) successfully cryopreserved the avian spermatozoa using glycerol as a cryoprotectant. Thereafter, cryopreservation of male gamete became possible. Blaxter (1953) applied a similar approach for fish gametes and reported success with Atlantic herring spermatozoa, achieving approximately 80% cellular motility after thawing. Since then, cryopreservation of fish sperm has been studied and has been successful in more than 200 species (Kopeika et al. 2007; Tiersch et al. 2007; Tsai et al., 2010) and techniques of sperm management have been established for freshwater and marine fish species, including carp, salmonids, catfish, cichlids, medakas, white-fish, pike, milkfish, grouper, cod, and zebrafish (Scott and Baynes 1980; Harvey and Ashwood-Smith 1982; Stoss and Donaldson 1983; Babiak et al. 1995; Suquet et al. 2000; Van der Straten et al. 2006; Bokor et al. 2007; Tsai et al. 2010). Many studies on cryopreservation of fish sperm have been carried out on economically important freshwater species and attempts to cryopreserve sperm from the marine fish species tended to be more successful when compared with those obtained from the freshwater fish (Tsvetkova et al. 1996). Although freshwater fish sperm are generally more difficult to cryopreserve, the fertilization rates obtained from the cryopreserved marine fish sperm are similar to those obtained with mammalian species (Tsvetkova et al. 1996). Controlled-rate slow cooling in cryopreservation has been mainly used for fish sperm. Common carp has been studied using frozen-thawed sperm with 95% fertilization and hatching rate.

Salmonid species spermatozoa have been successfully cryopreserved (Lahnsteiner 2000). Another well studied cryopreserved group is cyprinids and some of these cyprinid fishes are widely farmed throughout Asia and Europe. A fertilization and hatching rate of 95% using the

frozen-thawed sperm has been reported for the common carp and these results are not significantly different from fresh sperm (Magyary et al. 1996). Tilapias are among the exotic freshwater fishes that have been successfully established for fish farming in Taiwan; they have been cryopreserved successfully and produced 40-80% motility with cryoprotectant DMSO (Chao et al. 1987). The sperm of more than 30 marine fish species have been cryopreserved successfully (Suquet et al. 2000; Gwo 2000; Van der Straten et al. 2006). Generally, high survival and fertilization capacity has been obtained in frozen-thawed spermatozoa when compared to freshwater species (Drokin 1993; Gwo 2000).

Successful cryopreservation of the sperm of aquatic invertebrate has been carried out for sea urchin, oyster, starfish, abalone and coral (Adams et al. 2004a; Adams et al. 2004b; Gwo et al. 2002; Hagedorn et al. 2006; Kang et al. 2009). Dimethyl sulfoxide has also been reported as a successful cryoprotectant for sperm cryopreservation; the concentration range used was 5 to 30% for these species. Various levels of motility, ranging from <5% to 95%, have been reported for the cryopreserved aquatic invertebrate sperm (Dunn and McLachlan 1973).

Embryos

Cryopreservation of embryos has become an integral part of assisted reproduction. Successful cryopreservation of embryos is important because the biodiversity of both the paternal and maternal genomes will be preserved. While cryopreservation techniques have been largely established for the mammalian embryos, successful cryopreservation of intact fish embryos has not yet been achieved. Factors limiting fish embryo cryopreservation include their multicompartmental biological systems, high chilling sensitivity, low membrane permeability and their large size, which gives a low surface area to volume ratio (Zhang and Rawson 1995). The effect of such low ratio is a reduction in the rate at which water and cryoprotectants can move into and out of the embryo during cryopreservation (Mazur 1984). Fish embryos are osmoregulators; they are released into the external medium and activated. Then the vitelline envelope separates from the plasma membrane and forms chorion. Studies on the chorion permeability of zebra fish embryos clearly showed that it was permeable to electrolytes and a range of cryoprotectant, including propane-1,2-diol, methanol, DMSO, ethylene (Zhang and Rawson 1996). The chorion structure plays a crucial role as flexible filter for the transport of some materials (Toshimori and Tsuzumi 1976) and protects against the microorganisms (Schoots et al. 1982) Studies on zebra fish embryos have shown that the water permeability of the plasma membrane at different developmental stages remained relatively stable. The permeability to methanol (cryoprotectant) appeared to decrease during embryo development (Zhang and Rawson 1998). This also indicated that there was a gradual reduction in the permeability following the fertilization in zebra fish embryos, as opposed to the generally held belief that the membrane permeability of fish embryos reduced rapidly to minimum shortly after the fertilization (Alderdice 1988).

The studies on the kinetics of subzero chilling injury in *Drosophila* embryos (Mazur et al. 1992) and chilling sensitivity of zebra fish embryos have demonstrated that chilling injury plays an important role in reduction of embryo survival during the exposure to subzero temperatures (Zhang and Rawson 1995; Hagedorn et al. 1997). Chilling sensitivity has been shown for many species and has been analyzed in fish embryos, including brown trout (*Salmo trutta f. fario*)

(Maddock 1974), rainbow trout (*Oncorhynchus mykiss*) (Haga 1982), carp (*Cyprinus carpio*) (Dinnyes et al. 1998), fathead minnows (*Pimephales promelas*) (Cloud et al. 1988), goldfish (*Carassius auratus*) (Liu et al. 1993) and zebrafish (*Danio rerio*) (Zhang and Rawson 1995; Zhang et al. 2003). These studies demonstrated that the later stages (after 50% epiboly) were less sensitive to chilling, but chilling sensitivity increased significantly as the temperature fell below zero. The high chilling sensitivity of fish embryos, especially at early stages, their complex membrane structure and large yolk are the main obstacles to achieve successful cryopreservation of these embryos (Zhang and Rawson 1996). Chilling injury in embryos has been linked to the inhibition of metabolic and enzymatic processes from low temperatures injuries which could be detrimental in the embryonic development such as fish embryos (Dinnyes et al. 1998). Cryoprotectant toxicity follows a similar pattern to chilling sensitivity with later stages being less sensitive to cryoprotectant (Zhang et al. 2005; Zhang et al. 1993; Liu et al. 1993; Suzuki et al. 1995). Several studies have determined membrane permeability for zebra fish embryos (Zhang and Rawson 1998; Hagedorn et al. 1997) and membrane permeability to water and most cryoprotectants has been shown to be low (Zhang and Rawson 1996; Zhang and Rawson 1998). Studies on the cryopreservation of zebra fish embryos demonstrated 8% embryo survival in 2M methanol at -25 °C; however, no embryo survival was observed when frozen to -30 °C or below (Zhang et al. 1993).

Cryopreservation studies on the embryos and larvae have been conducted on marine invertebrate such as oysters, sea urchins, polycheate worms, coral and penaeid shrimp species (Liu et al. 2001; Gakhova et al. 1988; Lin et al. 1999; Olive and Wang 1997; Paniagua-Chavez and Tiersch 2001; Hagedorn et al. 2006; Tsai and Lin 2009). However, survivals of most of these species has been inadequate in maintaining the structure and activity of embryos and larvae after freezing to cryogenic temperatures. Embryonic and larval development of marine invertebrates after cryopreservation often showed abnormalities in structure and colour (Odintsova et al. 2001). The problems with invertebrate embryo cryopreservation associated with those identified with the fish embryos are their low membrane permeability and high chilling sensitivity. Although cryopreservation of the embryos has not been fully achieved, considerable progress has been made in understanding the conditions required for fish embryo cryopreservation and this would undoubtedly assist the successful protocol design in the future.

Oocytes

Oocyte cryopreservation is potentially the best way to preserve the female fertility. Cryopreservation of fish oocyte has been studied (Isayeva et al. 2004; Plachinta et al. 2004; Zhang et al. 2005; Guan et al. 2008; Tsai et al. 2009) which offers several advantages such as the smaller sizes range, much lower water content in oocytes and absence of a fully developed chorion that the permeability to water and solutes in oocyte is higher than embryo. Fish embryos are too large to apply traditional cryopreservation protocol. Immature oocytes can be an alternative for the mature eggs because of their smaller size (Hagedorn et al. 1996). However, there is no practical technique available to induce the small oocyte to mature *in vitro*. A technique to obtain the mature eggs from the late stage oocytes is available. Thus, the combination of this technique and their cryopreservation could be a breakthrough. However, at present, late stage oocytes cannot be successfully cryopreserved because their size is still not small enough to result in much lower surface area to volume ratio. These reduce the rate at which

water and cryoprotectant move into and out of oocytes during the cryopreservation. Developing the methods for cryopreservation of oocytes requires the screening of potential cryoprotectant treatments, evaluation of tolerance to chilling, determination of the appropriate rate of freezing to cryogenic temperatures and rate of thawing. Viability assessment methods of oocytes with trypan blue (TB), fluorescein diacetate (FDA) + propidium iodide (PI) and adenosine triphosphate (ATP) content assay have been developed for quick assessment of viability (Plachinta et al. 2004; Zampolla et al. 2006; Guan et al. 2008; Tsai et al. 2008; Tsai et al. 2009; Tsai et al. 2011; Tsai and Lin 2012). A functional test based on *in vitro* maturation, followed by germinal vesicle breakdown (GVBD) has also been shown effective for late stage III oocyte (Plachinta et al. 2004).

The permeability of the zebra fish oocyte membrane to water and cryoprotectants has been studied (Zhang et al. 2005) and membrane permeability was shown to decrease with the temperature and permeability was generally lower than those obtained from sea urchin eggs (Adams et al. 2003) but higher than the immature medaka oocyte (Valdez et al. 2005). Studies on zebra fish oocyte chilling sensitivity showed that those oocytes were very sensitive to chilling and their survival decreased with decreasing temperature (Isayeva et al. 2004). Chilling sensitivity in zebra fish oocytes was thought to be due to lipid phase transition of the oocyte membrane (Pearl and Arav 2000). The phase transition in zebra fish oocytes showed that chilling injury could occur when oocytes were exposed to temperatures between 12 to 22°C above the water freezing temperatures (Drobnis et al. 1993; Pearl and Arav 2000). Cryopreservation of late stage zebra fish oocytes has been studied using the controlled slow cooling and an optimum cryoprotective medium and cooling rate identified. Guan et al. (2008) showed that although the oocyte viability obtained immediately after freeze-thawing was relatively high with 88% using TB staining; oocyte viability decreased to 29.5% after 2 h incubation at 22 °C. The study also showed that the ATP level in the oocytes decreased significantly after thawing and all oocytes became translucent. Cryopreservation of early stage zebra fish oocytes using the controlled slow freezing has been reported by Tsai et al. (2009). The results suggested that 4M methanol in KCl buffer was identified as the optimum cryoprotective medium. Although results obtained after the cryopreservation using trypan blue and FDA+PI staining were promising with 69% and 54%, especially with stage II ovarian follicles, the ADP/ATP ratio assay showed that the energy system of these follicles had been compromised. Apparently the ADP/ATP ratio could be a valuable measure of cellular injury after post-thaw incubation period as it reflected the metabolic and energy status of population as well as indicating some measure of the potential for repair. Furthermore, *in vitro* culture method is effective for assessing early stage zebra fish oocytes growth competence *in vitro.* The early stage zebra fish oocytes can be cultured *in vitro* for 24 h, stage I and II oocytes can grow to the sizes of early stage II and stage III oocytes after hCG treatment. and also can be used for other teleost species (Tsai et al. 2010).

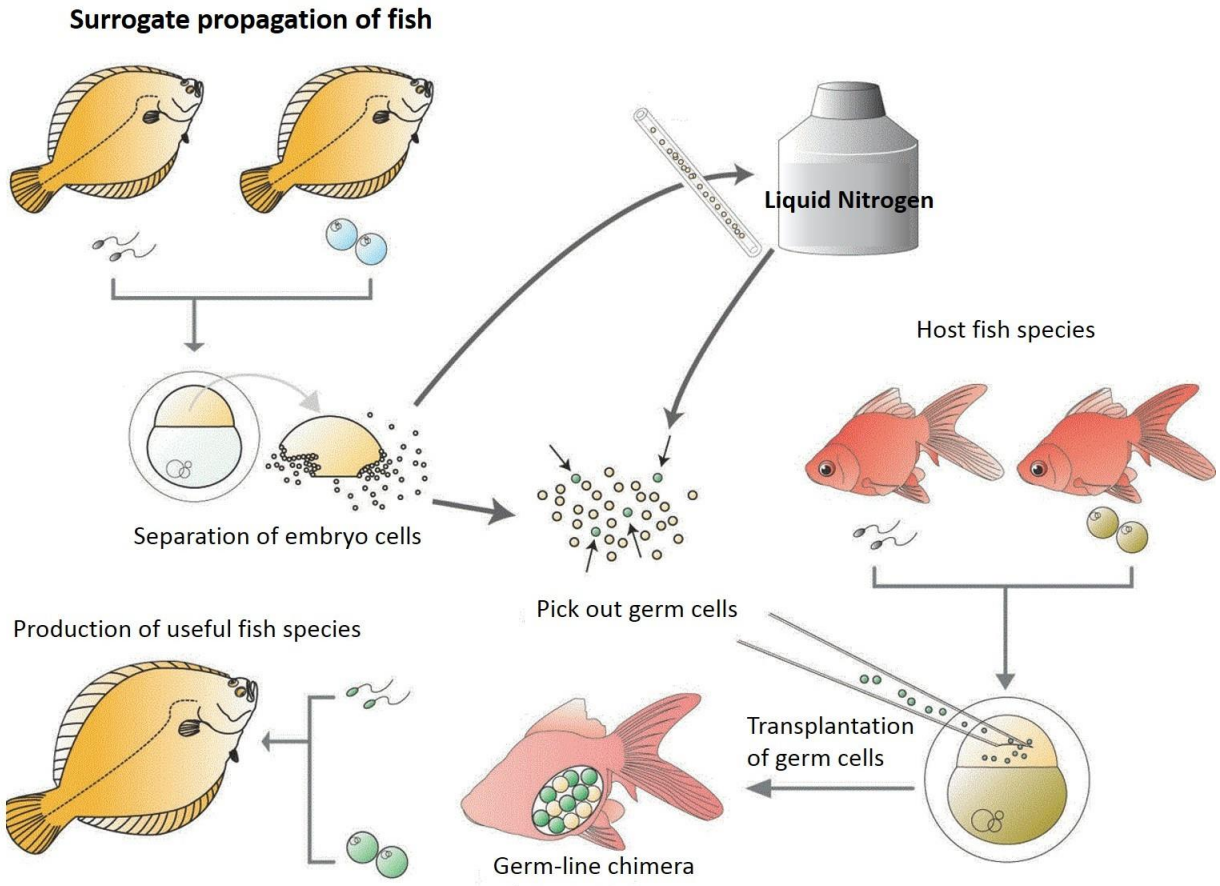
Studies on the cryopreservation of invertebrate oocytes and eggs over the past several decades have been extraordinarily difficult to achieve (Koseoglu et al. 2001; Tsai et al. 2010; Lin et al. 2011; Lin and Tsai 2012). However, it was found that intracellular crystallization occurred in the starfish oocytes at relatively high temperature that was very close to the temperature of extracellular ice formation (Koseoglu et al. 2001). In order to avoid this problem, Hamaratoglu et al. (2005) successfully cryopreserved starfish oocytes using ultra-rapid freezing technique, called vitrification. High chilling sensitivity (Tsai et al. 2009) and low membrane permeability (Guan et

al. 2008) of zebra fish oocytes are major obstacles to the development of a successful protocol for their cryopreservation as chilling sensitivity or cold shock can hinder slow cooling processes. Vitrification may be another option to achieve successful cryopreservation for the oocytes.

Blastomeres

Blastomeres are the cells produced as the result of cell division and cleavage in the fertilized egg. They are totipotent and pluripotent (depending on the stage of embryonic development) having the ability to differentiate into any of the three germ layers or entire organism. They are different from the muscle cells, blood cells or nerves cells. Although cultured somatic-cells from fish have been cryopreserved successfully, their value is limited because of loss of development potential. Cryopreservation of blastomeres can maintain the genetic diversity of both, nuclear genome and mitochondrial DNA (Nilsson and Cloud, 1992). Blastomeres from the early embryos of fish still retain pluripotency (Ho and Kimmel 1993) and their cryopreservation may be a promising approach to preserve the genotypes of zygotes and reconstitution of the organism. Indeed, there are several reports of germ-line chimeras created using the transplantation of blastomeres into goldfish (Yamaha et al. 1997; Kusuda et al. 2004), zebra fish (Lin et al. 1992), medaka (Hong et al. 1998; Wakamatsu et al. 2001) and rainbow trout (Takeuchi et al. 2001) embryos. Kusuda et al. (2004) transplanted the frozen-thaw blastomeres into goldfish embryos and the blastomeres differentiated into primordial germ cells. This report demonstrated that germ-line cells from the cryopreserved blastomeres could develop into mature gametes of chimeric fish because the blastomeres were not damaged by cryopreservation. Therefore, the cryopreservation techniques are very important.

Cryopreservation of blastomeres has been successful in several fish species. In the first reported studies, Harvey (1983) used a two-step freezing procedure, with ice-seeding at -6°C, and cooling to -25°C, followed by immersion in liquid nitrogen. The survival rate of 84.8% was obtained after cryopreservation of 50% epiboly zebra fish blastomeres. However, the results obtained from a very small sample size and freezing rates were not controlled, rather tubes were allowed to equilibrate in the cooled alcohol baths. Lin et al. (2009) demonstrated the effect of cryopreservation on zebra fish blastomeres survival using the controlled slow cooling method. It was shown that DMSO was the most toxic to zebra fish blastomeres. However, DMSO was the best cryoprotectant in terms of survival of zebra fish blastomeres. Therefore, it is possible that the cryoprotective effect of DMSO may be greater than its toxicity effect. Although the survival rate in Lin's results progressed from 25% (Kopeika et al. 2005) to 70%, it was still lower than that obtained by using two step methods. The comparisons between these studies must take into consideration the different methodology. Vitrification of zebra fish blastomeres was studied more recently and the highest blastomere survival was 93.4% (Cardona-Costa and Garcia-Ximénez 2007). Cryopreservation of blastomeres was also carried out in rainbow trout, carp and medaka after post-thawing. Rainbow trout blastomeres have been cryopreserved using the controlled slow freezing procedures with a survival of 95% (Calvi and Maisse 1998). It has been reported that the controlled slow freezing protocol adopted for rainbow trout was successfully applied to carp blastomeres with survivals of 94% and 96% (Calvi and Maisse 1999). Lower survival rates of cryopreserved blastomeres using controlled slow freezing have also been reported for other fish species such as whiting (20%), medaka (34%), pejerrey (67%) and chum salmon (59%) (Strussmann et al. 1999; Kusuda et al. 2002).



CONCLUSION

Cryopreservation of gametes and embryos are already routinely applied in the mammalian. Cryopreserved sperm, oocytes and embryos are used for artificial insemination and embryo transfer in the livestock industry. Cryopreservation also has enormous applications in the artificial propagation of widely diverse aquatic organism. Sperm and embryonic cells cryopreservaiton has been successful in a number of teleosts and invertebrate species. However, cryopreservation of embryos and oocytes remain a major challenge. The practical application of cryopreservation in the aquatic species needs more vigorous research efforts in this area and the efforts may be prioritized on endangered, economical value and representative species from various aquatic habitats. Cryopreservation of gametes, embryos and embryonic cells has become of immense value in aquatic biotechnologies which provide an important tool for protecting the endangered species, genetic diversity in aquatic species. The establishment of cryobanks to utilize the cryopreservation worldwide would be a significant and promising task in the future.

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Unit-II

Part-A

1. Define Aquaculture.
2. Write short notes on Semi-Intensive culture?
3. Enlist any four Lobster species.
4. Narrate Androgenesis.
5. Comment on Eye-stalk ablation.

Part-B

1. Write an illustrate account on sea bass culture?
2. Write an elaborate account on Crab culture?
3. Write an essay on tiger shrimp culture?
4. How would you produce pearls from oyster? Explain with suitable diagrams.
5. How would you cultivate seaweeds? Explain.
6. Write an elaborate account on fish genetics?

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.Tech. BIOTECHNOLOGY

UNIT – III - MARINE BIOTECHNOLOGY – SBT1304

UNIT – III

Biomedical Importance of Marine Organisms

Screening for new metabolites from marine microorganisms - Production of marine microalgae – Bio fuel production- Marine enzymes - Production of omega-3 fatty acids from marine organisms. Marine pharmacology: New antibiotics and medicines from marine organisms. Secondary metabolites from marine bacteria, actinomycetes and marine endophytic fungi– Probiotics.

SCREENING AND NEW METABOLITES FROM MARINE MICROORGANISMS

MARINE MICRO ORGANISMS For centuries, higher plants are major sources of drug used in many civilizations since ancient times, although the nature of the compounds in the drug is not exactly known. After the discovery of penicillin, attention has been focused on searching from terrestrial microorganism to look for new sources of drug and many new families of antibiotics are found from these microorganism. Marine microbes having immense genetic and biochemical diversity look likely to become a rich source of novel effective drugs. Marine bacteria constitute ~ 10% of the living biomass carbon of the biosphere32 and they represent dramatically different environment than their terrestrial counterpart. These bacteria originate mainly in sediments but also occur in open oceans and associated with the marine organisms. It was surprising to find that many bioactive compounds, reported from marine invertebrates are produced by their microbial symbionts. Competition among microbes for space and nutrients in the marine environment is a driving force behind the production of such precious antibiotics and other useful pharmaceuticals. Interestingly microorganisms associated with marine invertebrates are proved valuable candidates for drug discovery program33-35 Like bacteria, marine fungi are also reported to be potential source of bioactive substances. Sorbicilactone-A, novel type alkaloids was reported from sponge (Ircinia fasciculata) associated fungus, Penicillium chrysogenum. This compound showed therapeutic human trials. Polyketide synthases (PKSs) are a class of enzymes that are involved in the biosynthesis of secondary metabolites such as Erythromycin, Rapamycin,

Tetracycline, Lovastatin and Resveratrol. Polyketide biosynthetic genes from bacteria and fungi have been cloned, sequenced and expressed in heterologous hosts. Some marine sponge

associated bacteria with antimicrobial assets are also detected to have polyketide synthases gene cluster and investigation is underway to explore them. Deep-sea hydrothermal vent microorganisms are also reported to produce unusual bioactive metabolites. Symbionts Sponges are filter feeders, not completely sealed off from the surrounding medium. This may facilitate the formation of various types of associations with other organisms; some of these associations with other organisms; some of these associations may be more permanent than others. They can be intracellular as well as extracellular although fitness effects and the permanence of these relationships remain largely unknown.27 The presence of large amounts of micro-organisms within the mesophyl of many demosponges is well documented. Bacteria are probably permanently associated with the host sponge unless they are disturbed by external stress factors. Several recent studies have sought to address the phylogenetic diversity of microbial communication associated with marine sponges by using 16 sRNA gene sequence analysis. A comprehensive analysis showed that sponges from different oceans contain phylogenetically complex, yet highly specific, microbial signatures. In particular, representatives of the poorly characterized phyla chloroflexi, Acidobacteria and Actinobacteria are abundant in gene libraries.36 Micro-organisms associated with marine invertebrates are reported to be involved in the production of bioactive molecules. Bioactive compound production in these bacteria could be attributed to the competition among them for space and nutrition. Though these bioactive compounds may be important for epibiotic defense of marine invertebrate hosts, they also have significant medical and industrial applications.35,37 Marine sponges and the microbes using within them are important from both an ecological viewpoint sponges are important members of shallow and deep water reef communication with nutrition supplied by photosynthetic symbionts often allowing them to compete with other benthic organisms such as corals. In some cases the active metabolites are produced by the microbes, rather than the sponge itself. Sponges and their associated microorganisms are therefore receiving much attention from pharmaceutical companies.38 Convincing evidence for the involvement of micro organisms in natural product synthesis has been complied for the tropical sponges Dysidea habacea and Theonella swinhoei, in which the producing microbe is a cynobacterium in the former and a bacterium in the latter.39 Sponge's harbor a rich diversity of micro organism in their tissues and in some case constitute up to 40% of the biomass, e.g. the Mediterranean sponge Aplysina aerophoba.40,41 Sponge associated bacteria are capable of producing antibacterial metabolites. Surface associated

bacteria with sponge Ircinia ramosa has shown Antibacterial activity.14 Several bacteria activated from tunicate have yielded natural products. An example is andrimid and the moramides A-C from a Pseudomonas flurescens strain, Harman previously known from tunicates and was shown also to be synthesized by a tunicate – associated Enterococeus faecium. The antifouling agent six bromoindole – 3 – baldehyole and its debromo derivative were isolated from the ascidian Stomozoa murrayi and also from an Actinobacter Sp. associated with this animal .42 The epibiotic bacteria in seaweed play a protective role by releasing secondary metabolites into the surrounding seawater that help preventing extensive fouling of the surface. Epibiotic bacteria are therefore attracting attention as a source of new natural products.43 The proportion of active bacteria associated with marine invertebrates (20%) and seaweeds (11%) is higher than that isolated from seawater (7%) and sediment (5%) .

PRODUCTION OF MARINE MICROALGAE

Introduction

The early life stages of seabass and gilthead seabream are zooplankton-feeders, i.e. they prey on small free living planktonic animals. As no artificial larval diet can at present totally fulfil their nutritional requirements, their successful rearing still depends on an adequate supply of high quality live feeds, usually in the form of rotifers (fed on unicellular algae) and brine shrimp *(Artemia spp).*

Mass culture of microalgae

Mass production of phytoplankton for rotifers and "green water" in most Mediterranean hatcheries is limited to a few species such as: *Chlorella sp, Isochrysis galbana, Pavlova lutheri, Nannochloropsis oculata* and *N. gaditana, Dunaliella tertiolecta* and *Tetraselmis suecica.* These species have been selected on the basis of their size, nutritional value, culture easiness and absence of negative side effects, such as toxicity. Their nutritional value shows a great variability not only among different species, but also in genetically different populations of the same species (strains). For hatchery purposes, the species to be cultured should both fit well the local rearing conditions and have a high nutritional value for rotifers. The increasing availability of nutritional boosters as enrichment diets for both rotifers and brine shrimps, has made this choice easier.

Fig. 23.01 Mass culture of microalge (photo STM Aquatrade)

Population dynamics

Microalgae population dynamics can be described by different phases:

- the lag-phase, where, just after the inoculum, the cells increase in size, but not in number, and begin to absorb the nutriens supplied with the culture medium;
- the log-phase (or esponential phase), where cells reproduce very fast and population growth is exponential;
- the transitional phase (or declining growth phase), where growth rate slows down;
- the stationary phase, where cells remains constant in number and reproduction is balanced by death;
- the decline phase, where cell number decreases since death rate exceeds growth.

It is advisable to harvest phytoplanktonic organisms during their log phase, since in the new culture they will grow more rapidly and will yield a more viable population.

Mass production systems

For aquaculture purposes, microalgae are mass produced in three main ways: (i) batch (or discontinuous or multistep back-up system) culture, (ii) semi-continuous culture, and (iii) continuous culture.

In the batch culture a small axenic stock culture produces a series of cultures of increasing volume where the algal population of each culture vessel is entirely harvested at or near its peak density, i.e. while still conserving a good growth potential, to be used either as inoculum for other culture vessels, or to feed rotifers or be used in fish larval tanks. It typically makes use of small (few liters) to medium size (500 liters) containers, and it is kept indoor and under strictly controlled, if not properly axenic, conditions. It is considered by many authors the easiest and most reliable method of algal production, provided that the working protocol is strictly enforced.

Algal quality is less erratic than in the semi-continuous method, even if the latter is more productive for any given volume.

In the semi-continuous system the algal population, when mature, is partially harvested at intervals. The harvested culture volume is replaced by fresh medium to keep growth going on. This culture is adopted to produce large amounts of algae and frequently uses large outdoor tanks. Their main drawbacks are: (i) the unpredictable duration, (ii) the risk of contamination by other organisms as competitors (other microalgal species), contaminants (bacteria) and predators (ciliate protozoa feeding on the algae), as well as (iii) the building up of metabolites, which can affect quality.

The continuous system is a steady-state continuous flow culture in which the rate of growth is governed by the rate of supply of the limiting factor. It is a balanced axenic system where the algal population is harvested and fertilised continuously. This method, though the most efficient over extended periods, produces limited amounts of high quality cells and requires complex equipment as well as advanced management. A relatively recent development of this system is represented by the photo-bioreactor, a continuous culture device that increases the density of cultured microalgae to very high levels under predictable environmental and microbiological conditions.

The microalgae produced can be concentrated to a dense liquid suspension by centrifugation, and can then be stored for more than one month in the refrigerator, still giving excellent viability when used. A new industry is now appearing, whose concentrated algal products can also fulfil the hatchery needs, saving the time-consuming and expensive production of microalgae in the hatchery.

The system described below is the batch culture, by far the most widely adopted method by Mediterranean hatcheries. Before its description, additional instructions are given concerning facilities, the preparation of the culture medium, and the equipment required.

Fig. Old fashioned unit using artificial light for algae mass culture (photo M. Caggiano)

Mass culture facilities for microalgae

Algae are cultured in a dedicated sector of the live feeds production section, which is made of three working areas inside the hatchery building: a lab for duplicating small cultures, a conditioned room to maintain small culture vessels and pure strains and finally a large area for the mass cultures in PE bags or, less frequently, tanks. In the warmest Mediterranean areas, a light greenhouse can replace the latter.

Small volume cultures are kept in vessels ranging from 20-ml test tubes up to 18 l carboys. They can be made of borosilicate glass, polycarbonate, PET or any other material able to stand a sterilization process. These vessels are placed on glass shelves lightened by fluorescent tubes and equipped with a CO_2 enriched air distribution system.

Hot-extruded tubular PE film is utilised for larger volumes bags. The film is usually 0.25 mm thick and its stretched width ranges from 45 to 95 cm. Two bag designs are widely adopted in Mediterranean hatcheries: the smaller suspended bag and the larger one placed within a steel wire cylindrical frame. The first type has a capacity of 60 I (single) to 150 I (double or U-shaped), whereas the latter, that stands on a saddle-like GRP base to improve circulation, can contain up to 450l. Their top is closed by a plastic cover to prevent contamination.

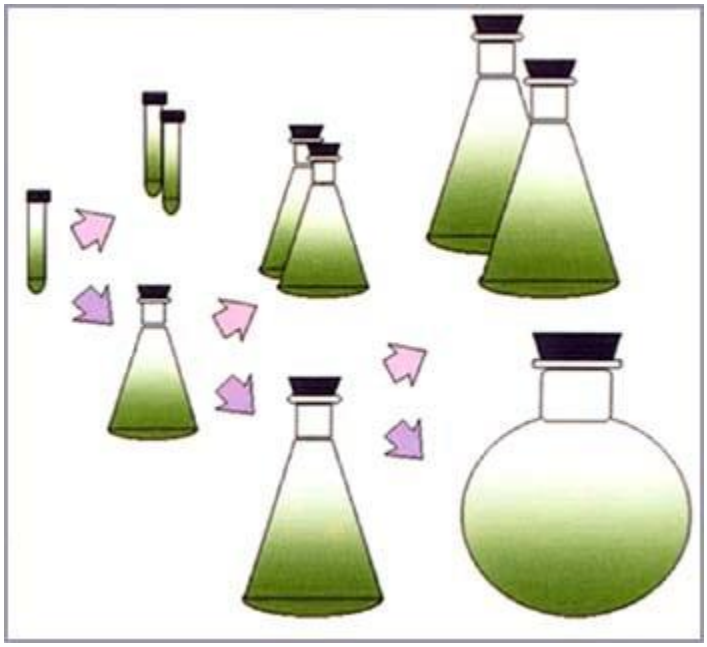

Fig 24.00 A typical scheme of a batch type production

All units are equipped with artificial lights, usually fluorescent tubes, an aeration system, often with an additional source of carbon dioxide, and stands for the culture vessels, i.e. light shelves for small volumes and metal racks or wired frames for PE bags.

The unit also stores the special equipment to process pre-treated seawater, such as fine filters and sterilizers, as well as a laboratory where nutrients and glassware are prepared and stored, and

where the necessary monitoring operations are performed. Standard cleaning procedures have to be strictly followed to maintain proper hygienic conditions

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BIOFUEL (Biodiesel) production from marine microalgae *Chlorella marina* & *Nannochloropsis salina*

Mechanical energy cannot be achieved successfully without petroleum, natural gas, coal, hydro electricity and nuclear energy; and they became the basic natural sources for the energy. The demand of petroleum and its by-products are increasing continuously due to the increase in population and industrialization. The discriminate use of petroleum sourced fuels is now widely recognized as unsustainable because it is non-renewable resources. In the last 10 years, many studies have been conducted on biofuels for substituting fossil fuels and reduce the greenhouse gas (GHG) emission which is responsible for global warming (Bastianoni et al., 2008). Biodiesel production from microalgae is an emerging technology considered by many as a very promising source of energy, mainly because of its reduced competition for land. Among these, especially, microalgae were found to be an alternative nature source of renewable petroleum resources that is capable of meeting the global demand for fuels (Chisti, 2007, 2008). The idea of using algae as a source of fuel is not new (Chisti, 1981; Nagle and Lemke, 1990; Sawayama et al., 1995), but it is now being taken seriously because of the increasing price of petroleum and more significantly, the emerging concern about global warm that is associated with burning fossil fuels (Gavrilescu and Chisti, 2005). It is reported that microalgae can provide several different types of renewable biofuels which include, methane, biodiesel and biohydrogen (Gavrilescu and Chisti, 2005; Kapdan and Kargi, 2006; Spolaore et al., 2006). Microalgae have short life cycle and use a photosynthetic process similar to higher plants for their energy. In fact, the biomass doubling time for microalgae during exponential growth is found as short as 3.5 h. Microalgae are veritable miniature biochemical factories, and appear photosynthetically more efficient than terrestrial plant, and are efficient CO2 fixer (Pirt, 1986). The ability of algae to fix CO2 has been proposed as a method of removing CO2 from fuel gases from power plants, and thus, can be used to reduce emission of GHG (Chisti, 2007). Many algae are exceedingly rich in oil, which can be converted to biodiesel. The oil content of some microalgae exceeds 80% of dry weight (DW) of algae biomass (Banerjee et al., 2002; Chisti, 2007). Microalgae are faster in growth in

the marine environment and yield of oil from algae is estimated between 5000 to 20000 m 3 / 4046 m 2 /yr which is 7 to 31 times greater than the terrestrial crop, palm oil (635 m 3) (Pringsheim, 1950). The high growth rate of microalgae makes it possible to satisfy the massive demand on biofuels using limited land resources. Microalgae cultivation consumes less water than land crops. Most microalgae biomass contains three main components such as 1) lipids, 2) proteins, and 3) carbohydrates and/or hydrocarbons. Microalgae produce and store lipids in the form of fatty acids, phospholipids, glycolipids and it can be used as feedstocks for biodiesel production by transesterification reaction in the presence of acid or base with methanol.

Compared with terrestrial crops which take a season to grow and only contain a maximum of about 5% DW of oilmicroalgae, grow quickly and contain high oil content. This is why microalgae are the focus in the algae-tobiofuel arena. Oil content of microalgae is usually between 20 and 50%, while some strains can reach as high as 80%. Hence, the present study was made on culture of two different microalgae, growth, flocculation activities, oil content and identification by using ASTM standards. The results obtained from this investigation revealed that N. salina and C. marina were easy to cultivate which contains high lipid content. The faster growth rate as well as higher oil content found with these microalgae will make these as the potential candidate for alternative biodiesel production.

MARINE ENZYMES

Marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptivity, and ease in largescale cultivation. This review deals with the research and development work done on the occurrence, molecular biology, and bioprocessing of marine enzymes during the last decade. Exotic locations have been accessed for the search of novel enzymes. Scientists have isolated proteases and carbohydrases from deep sea hydrothermal vents. Cold active metabolic enzymes from psychrophilic marine microorganisms have received considerable research attention. Marine symbiont microorganisms growing in association with animals and plants were shown to produce enzymes of commercial interest. Microorganisms isolated from sediment and seawater have been the most widely studied, proteases, carbohydrases, and peroxidases being noteworthy. Enzymes from marine

animals and plants were primarily studied for their metabolic roles, though proteases and peroxidases have found industrial applications. Novel techniques in molecular biology applied to assess the diversity of chitinases, nitrate, nitrite, ammonia-metabolizing, and pollutant-degrading enzymes are discussed. Genes encoding chitinases, proteases, and carbohydrases from microbial and animal sources have been cloned and characterized. Research on the bioprocessing of marine-derived enzymes, however, has been scanty, fo cusing mainly on the application of solid-state fermentation to the production of enzymes from microbial sources.

In the past decade, of the plentiful reports on enzymes from novel and exotic sources few have reached the stage of commercial production. The problemlies in providing the enzyme producers with the proper environmental conditions of their ecological niches. Sustained production of the bioactive molecules by novelmolecular methods of gene cloning and expression and innovative bioreactor designs like the so-called “niche-mimic” bioreactors [206] should play apivotal role.Marine enzymebiotechnologywillbe the focusof the industry in the future.Withmutual respect for each other’s commercial interests and intellectual property rights, the biodiverse but resource-poor developing world and the wealthy but bioresource-scarce developed world should join hands to unravel the secrets of this unopened research treasure chest—the world’s oceans.

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MARINE LIPIDS/FATTY ACIDS

Omega-3 fatty acids — also called **ω-3 fatty acids** or ***n*-3 fatty acids**[1] — are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the end of the carbon chain.[2] The fatty acids have two ends, the carboxylic acid (-COOH) end, which is considered the beginning of the chain, thus "alpha", and the methyl ($-CH_3$) end, which is considered the "tail" of the chain, thus "omega". The way in which a fatty acid is named is determined by the location of the first double bond, counted from the methyl end, that is, the omega (ω-) or the n- end.

The three types of omega-3 fatty acids involved in human physiology are α-linolenic acid (ALA) (found in plant oils), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (both commonly found in marine oils). Marine algae and phytoplankton are primary sources of omega-3 fatty acids. Common sources of plant oils containing the omega-3 ALA fatty acid include walnut, edible seeds, clary sage seed oil, algal oil,flaxseed oil, Sacha Inchi oil, *Echium* oil, and hemp oil, while sources of animal omega-3 EPA and DHA fatty acids include fish oils, egg oil, squid oils, and krill oil. Dietary supplementation with omega-3 fatty

acids does not appear to affect the risk of death, cancer or heart disease.[3][4]Furthermore, fish oil supplement studies have failed to support claims of preventing heart attacks or strokes.[5][6][7]

Omega-3 fatty acids are important for normal metabolism.[8] Mammals are unable to synthesize omega-3 fatty acids, but can obtain the shorter-chain omega-3 fatty acid ALA (18 carbons and 3 double bonds) through diet and use it to form the more important long-chain omega-3 fatty acids, EPA (20 carbons and 5 double bonds) and then from EPA, the most crucial, DHA (22 carbons and 6 double bonds).[8] The ability to make the longer-chain omega-3 fatty acids from ALA may be impaired in aging.[9][10] In foods exposed to air, unsaturated fatty acids are vulnerable tooxidation and rancidity.[11]

Health effects

Supplementation does not appear to be associated with a lower risk of all-cause mortality.[3]

Cancer

The evidence linking the consumption of fish to the risk of cancer is poor.[12] Supplementation with omega-3 fatty acids does not appear to affect this either.[4]

A 2006 review concluded that there was no link between omega-3 fatty acids consumption and cancer.[4] This is similar to the findings of a review of studies up to February 2002 that failed to find clear effects of long and shorter chain omega-3 fats on total risk of death, combined cardiovascular events and cancer.[13][14] In those with advanced cancer andcachexia, omega-3 fatty acids supplements may be of benefit, improving appetite, weight, and quality of life.[15] There is tentative evidence that marine omega-3 polyunsaturated fatty acids reduce the risk of breast cancer but this is not conclusive.[16][17]

The effect of consumption on prostate cancer is not conclusive.[17] There is a decreased risk with higher blood levels of DPA, but an increased risk of more aggressive prostate cancer with higher blood levels of combined EPA and DHA (found in fatty fish oil).[18]

Cardiovascular disease

Evidence, in the population generally, does not support a beneficial role for omega-3 fatty acid supplementation in preventing cardiovascular disease (including myocardial infarction and sudden cardiac death) or stroke.[3][19][20] However, omega-3 fatty acid supplementation greater than one gram daily for at least a year may be protective against cardiac death, sudden death, and myocardial infarction in people who have a history of cardiovascular disease.[21] No protective effect against the development of stroke or all-cause mortality was seen in this population.[21] Eating a diet high in fish that contain long chain omega-3 fatty acids does appear to decrease the risk of stroke.[22] Fish oil supplementation has not been shown to benefit revascularization or abnormal heart rhythms and has no effect on heart failure hospital admission rates.[23] Furthermore, fish oil supplement studies have failed to support claims of preventing heart attacks or strokes.[5][6][7]

Evidence suggests that omega-3 fatty acids modestly lower blood pressure (systolic and diastolic) in people with hypertension and in people with normal blood pressure.[24] Some

evidence suggests that people with certain circulatory problems, such as varicose veins, may benefit from the consumption of EPA and DHA, which may stimulate blood circulation and increase the breakdown of fibrin, a protein involved in blood clotting and scar formation.[25][26] Omega-3 fatty acids reduce blood triglyceride levels but do not significantly change the level of LDL cholesterol or HDL cholesterol in the blood.[27][28] ALA does not confer the cardiovascular health benefits of EPA and DHAs.[29]

The effect of omega-3 polyunsaturated fatty acids on stroke is unclear, with a possible benefit in women.[30]

Inflammation

Some research suggests that the anti-inflammatory activity of long-chain omega-3 fatty acids may translate into clinical effects.[31] A 2013 systematic review found tentative evidence of benefit.[32] Consumption of omega-3 fatty acids from marine sources lowers markers of inflammation in the blood such as C-reactive protein, interleukin 6, and TNF alpha.[33]

For rheumatoid arthritis (RA), one systematic review found consistent, but modest, evidence for the effect of marine n-3 PUFAs on symptoms such as "joint swelling and pain, duration of morning stiffness, global assessments of pain and disease activity" as well as the use of non-steroidal anti-inflammatory drugs.[34] The American College of Rheumatology (ACR) has stated that there may be modest benefit from the use of fish oils, but that it may take months for effects to be seen, and cautions for possible gastrointestinal side effects and the possibility of the supplements containing mercury or vitamin A at toxic levels. Due to the lack of regulations for safety and efficacy, the ACR does not recommend herbal supplements and feels there is an overall lack of "sound scientific evidence" for their use.[35] The National Center for Complementary and Integrative Health has concluded that "[n]o dietary supplement has shown clear benefits for RA", but that there is preliminary evidence that fish oil may be beneficial, and called for further study.[36]

Developmental disabilities

Although not supported by current scientific evidence as a primary treatment for ADHD, autism, and other developmental disabilities,[37][38] omega-3 fatty acid supplements are being given to children with these conditions.[37]

One meta-analysis concluded that omega-3 fatty acid supplementation demonstrated a modest effect for improving ADHD symptoms.[39] A Cochrane review of PUFA (not necessarily omega-3) supplementation found "there is little evidence that PUFA supplementation provides any benefit for the symptoms of ADHD in children and adolescents",[40]while a different review found "insufficient evidence to draw any conclusion about the use of PUFAs for children with specific learning disorders".[41] Another review concluded that the evidence is inconclusive for the use of omega-3 fatty acids in behavior and non-neurodegenerative neuropsychiatric disorders such ADHD and depression.[42]

Fish oil has only a small benefit on the risk of early birth.[43][44] A 2015 meta-analysis of the effect of omega-3 supplementation during pregnancy did not demonstrate a decrease in the rate

of preterm birth or improve outcomes in women with singleton pregnancies with no prior preterm births.[45] A systematic review and meta-analysis published the same year reached the opposite conclusion, specifically, that omega-3 fatty acids were effective in "preventing early and any preterm delivery".[46]

Mental health

There is some evidence that omega-3 fatty acids are related to mental health,[47] including that they may tentatively be useful as an add-on for the treatment of depression associated with bipolar disorder.[48] Significant benefits due to EPA supplementation were only seen, however, when treating depressive symptoms and not manic symptoms suggesting a link between omega-3 and depressive mood.[48] There is also preliminary evidence that EPA supplementation is helpful in cases of depression.[49] The link between omega-3 and depression has been attributed to the fact that many of the products of the omega-3 synthesis pathway play key roles in regulating inflammation such asprostaglandin E3 which have been linked to depression.[50] This link to inflammation regulation has been supported in both in vitro [51] and in vivo studies as well as in meta-analysis studies.[32] The exact mechanism in which omega-3 acts upon the inflammatory system is still controversial as it was commonly believed to have anti-inflammatory effects.[52]

There is, however, significant difficulty in interpreting the literature due to participant recall and systematic differences in diets.[53] There is also controversy as to the efficacy of omega-3 with many meta-analysis papers finding heterogeneity among results which can be explained mostly by publication bias.[54][55] A significant correlation between shorter treatment trials was associated with increased omega-3 efficacy for treating depressed symptoms further implicating bias in publication.[55]

There is some evidence to support the claim that omega-3 can help treat anxiety disorder symptoms as well but studies have been limited.[56]

Very low quality evidence finds that omega-3 fatty acids might prevent psychosis.[57]

Cognitive aging

Epidemiological studies are inconclusive about an effect of omega-3 fatty acids on the mechanisms of Alzheimer's disease.[58] There is preliminary evidence of effect on mildcognitive problems, but none supporting an effect in healthy people or those with dementia.[59][60][61]

Atopic diseases

Results of studies investigating the role of LCPUFA supplementation and LCPUFA status in the prevention and therapy of atopic diseases (allergic rhinoconjunctivitis, atopic dermatitis and allergic asthma) are controversial; therefore, at the present stage of our knowledge we cannot state either that the nutritional intake of n-3 fatty acids has a clear preventive or therapeutic role, or that the intake of n-6 fatty acids has a promoting role in context of atopic diseases.[62]

Dietary sources

Grams of omega-3 per 3oz (85g) serving[102][103]

Common name	grams omega-3
Flax	11.4 [104]
Hemp	11.0
Herring, sardines	1.3–2
Mackerel:Spanish/Atlantic/Pacific	1.1–1.7
Salmon	1.1–1.9
Halibut	0.60–1.12
Tuna	0.21–1.1
Swordfish	0.97
Greenshell/lipped mussels	0.95[104]
Tilefish	0.9
Tuna (canned, light)	0.17–0.24
Pollock	0.45

Grams of omega-3 per 3oz (85g) serving[102][103]

Common name	grams omega-3
Cod	0.15–0.24
Catfish	0.22–0.3
Flounder	0.48
Grouper	0.23
Mahi mahi	0.13
Orange roughy	0.028
Red snapper	0.29
Shark	0.83
King mackerel	0.36
Hoki (blue grenadier)	0.41[104]
Gemfish	0.40[104]
Blue eye cod	0.31[104]

Grams of omega-3 per 3oz (85g) serving[102][103]

Common name	grams omega-3
Sydney rock oysters	0.30[104]
Tuna, canned	0.23[104]
Snapper	0.22[104]
Eggs, large regular	0.109[104]
Strawberry or Kiwifruit	0.10-0.20
Broccoli	0.10-0.20
Barramundi, saltwater	0.100[104]
Giant tiger prawn	0.100[104]
Lean red meat	0.031[104]
Turkey	0.030[104]
Cereals, rice, pasta, etc.	0.00[104]
Fruit	0.00[104]

Grams of omega-3 per 3oz (85g) serving[102][103]

Common name	grams omega-3
Milk, regular	0.00[104]
Bread, regular	0.00[104]
Vegetables	0.00[104]

Daily values

In the United States, the Institute of Medicine publishes a system of Dietary Reference Intakes, which includes Recommended Dietary Allowances (RDAs) for individual nutrients, and Acceptable Macronutrient Distribution Ranges (AMDRs) for certain groups of nutrients, such as fats. When there is insufficient evidence to determine an RDA, the institute may publish an Adequate Intake (AI) instead, which has a similar meaning, but is less certain. The AI for α-linolenic acid is 1.6 grams/day for men and 1.1 grams/day for women, while the AMDR is 0.6% to 1.2% of total energy.[105]

A growing body of literature suggests that higher intakes of α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) may afford some degree of protection against coronary disease.[*citation needed*] Because the physiological potency of EPA and DHA is much greater than that of ALA, it is not possible to estimate one AMDR for all omega-3 fatty acids. Approximately 10 percent of the AMDR can be consumed as EPA and/or DHA.[105] There was insufficient evidence as of 2005 to set an upper tolerable limit for omega-3 fatty acids.[105]

Heavy metal poisoning by the body's accumulation of traces of heavy metals, in particular mercury, lead, nickel, arsenic, andcadmium, is a possible risk from consuming fish oil supplements.[*medical citation needed*] Also, other contaminants (PCBs, furans,dioxins, and PBDEs) might be found, especially in less-refined fish oil supplements.[*citation needed*] However, heavy metal toxicity from consuming fish oil supplements is highly unlikely, because heavy metals selectively bind with protein in the fish flesh rather than accumulate in the oil. An independent test in 2005 of 44 fish oils on the US market found all of the products passed safety standards for potential contaminants.[106][*unreliable source?*]

The FDA has advised that adults can safely consume a total of 3 grams per day of combined DHA and EPA, with no more than 2 g per day coming from dietary supplements.[107]

Throughout their history, the Council for Responsible Nutrition and the World Health Organization have published acceptability standards regarding contaminants in fish oil. The most stringent current standard is the International Fish Oils Standard.[108][*non-primary source needed*] Fish oils that are molecularly distilled under vacuum typically make this highest-grade, and have measurable levels of contaminants (measured parts per billion and parts per trillion).[*citation needed*]

A recent trend has been to fortify food with omega-3 fatty acid supplements. Global food companies have launched omega-3 fatty acid fortified bread, mayonnaise, pizza, yogurt, orange juice, children's pasta, milk, eggs, popcorn, confections, and infant formula.[*citation needed*]

The American Heart Association has set up dietary recommendations for EPA and DHA due to their cardiovascular benefits: Individuals with no history of coronary heart disease or myocardial infarction should consume oily fish or fish oils two times per week; those having been diagnosed with coronary heart disease after infarction should consume 1 g EPA and DHA per day from oily fish or supplements; those wishing to lower blood triglycerides should consume 2–4 g of EPA and DHA per day in the form of supplements.[103][*needs update*]

Fish

The most widely available dietary source of EPA and DHA is oily fish, such as salmon, herring, mackerel, anchovies, menhaden, and sardines. Oils from these fish have a profile of around seven times as much omega-3 as omega-6. Other oily fish, such astuna, also contain *n*-3 in somewhat lesser amounts. Consumers of oily fish should be aware of the potential presence of heavy metals and fat-soluble pollutants like PCBs and dioxins, which are known to accumulate up the food chain. After extensive review, researchers from Harvard's School of Public Health in the *Journal of the American Medical Association* (2006) reported that the benefits of fish intake generally far outweigh the potential risks. Although fish are a dietary source of omega-3 fatty acids, fish do not synthesize them; they obtain them from the algae (microalgae in particular) or plankton in their diets.[109]

Fish oil

Fish oil capsules

Marine and freshwater fish oil vary in content of arachidonic acid, EPA and DHA.[110] They also differ in their effects on organ lipids.[110] Not all forms of fish oil may be equally digestible. Of four studies that compare bioavailability of the glyceryl ester form of fish oil vs. the ethyl ester form, two have concluded the natural glyceryl ester form is better, and the other two

studies did not find a significant difference. No studies have shown the ethyl ester form to be superior, although it is cheaper to manufacture.[111][112]

Krill

Krill oil is a source of omega-3 fatty acids.[113] The effect of krill oil, at a lower dose of EPA + DHA (62.8%), was demonstrated to be similar to that of fish oil on blood lipid levels and markers of inflammation in healthy humans.[114] While not an endangered species, krill are a mainstay of the diets of many ocean-based species including whales, causing environmental and scientific concerns about their sustainability.[115][116][117]

Squid oil

Squid oil (also known as calamari oil) is another source of omega-3 fatty acid.[118] The editor of health365com.au considers squid environmentally friendlier than fish or krill oil, because it is prepared from the largely unused portions of squid catches.[119]

MARINE PHARMACOLOGY: NEW ANTIBIOTICS AND MEDICINES FROM MARINE ORGANISMS

Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug–resistant infectious disease and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of oceans. The marine resources are nowadays widely studied because of numerous reasons. One of the reason is as the oceans cover more than 70% of the world surface and among 36 known living phyla, 34 of them are found in marine environments with more than 300000+ known species of fauna and flora. 1-3 The rationale of searching for drugs from marine environment stem from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction. The attention of finding drug from sea had started from 1970s. For instance, about 300 patents on bioactive marine natural product have been issued between 1969 and 1999. So far, more than 10,000 compounds have been isolated from marine organisms.4 Only 10% of over 25,000 plants have been investigated for biological activity. The marine environment may contain over 80% of world's plant and animal species. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates, sponges, soft corals,

bryozoans, sea slugs and marine organisms.5 The marine environment covers a wide thermal, pressure and nutrient ranges and it has extensive photic & non-photic zones. This extensive variability has facilitated extensive specification at all phylogenetic levels, from microorganism to mammals. Despite the fact that the biodiversity in the marine environment for exceeds that of the terrestrial environment, research into the use of marine natural products as pharmaceutical agent is still in its infancy. This may be due to the lack of ethnomedical history and the difficulties involved in the collection of marine organisms.6 But with the development of new diving techniques, remote operated machines etc, it is possible to collect marine samples and during the past decade, over 4200 novel compounds have been isolated from shallow waters to 900-m depths of the sea.4 Several marine organisms are sessile and soft bodied, then the question will arise; how do these delicate looking simple sea creatures protect themselves from predators and pathogens in the marine environment. The answer to this inquest is the defense mechanism of the marine organism. The chemical compounds (like secondary metabolites) which are produced or obtained from micro organism. By the marine flora and fauna are very potent and biological active. The potency of bioactive from marine life is mainly due to the intensive ecology pressure and from the stronger and /or predators. Investigations in their chemical ecology have revealed that the secondary metabolites not only play various roles in the metabolism of the producer but also in their strategies in the given environment. The study on marine chemical compounds produced by different organisms; showed the strategies for their use for human benefit 7, 8, 9 To understand the link between marine chemical warfare and human health it is crucial to study chemical ecology in the oceans. Many sessile invertebrates such as sponges, corals and tunicates feed by filtering seawater. Since, seawater contains high concentrations of bacteria; these organisms produce antibiotics to defend themselves from potentially harmful microorganisms. Thus the production of anti-bacterial compounds by filter feeders such as sponges provides a possible link between chemical defense for sponges and antibiotics for use in humans. However, why should a sponge produce anticancer drugs or why a coral should produce a compound useful in the treatment of arthritis? In the scenario of two encrusting sponges growing together, the sponge that will win the race of competition for space is the one that produces the chemical most effective at killing the rapidly dividing cells of the neighboring sponge. The ability of chemical to kill rapidly dividing cells is the hallmark of chemotherapy. Anticancer drugs often act by killing the rapidly dividing cells of a tumor but

generally do not harm ‘normal’ healthy cells. These ideas provide a connection between marine chemical warfare and the possible application of marine natural products in medicine. Chemical ecology of marine organisms relates very closely to biotechnology by exploring these secondary metabolites to develop drugs to treat various life threatening diseases. Natural products released into the water is rapidly diluted and therefore need to be highly potent to have any effect. For this reason, and because of the immense biological diversity in the sea as a whole chemical entities exist in the ocean with biological activities that may be useful in the quest of finding drugs with greater efficacy and specificity for the treatment of many human diseases 4,10. It’s difficult to summarize the whole ocean wealth of life in one review, thus few major organisms discussed below: SPONGES Sponges are often studied because of their wealth of metabolites, which display biological activity. This is related to the nutritional physiology of these filter feeding animals, which efficiently filter bacteria from the inhalant water current. The diffusion of antibiotic agents in the living tissues may increase the efficiency of the retention mechanism concerned, and may also provide a defense against microbial infections and/or be used to control symbiotic bacteria populations. Inhibition and promotion of microbial growth by sponge extracts have been illustrated in simple experiments with laboratory and marine cultures of bacteria and of pathogenic fungi. However, their ecological and physiological significance remains largely unknown.11 So far an estimated 15,000 species have been described, but the true diversity is probably much higher. Particularly the tropical sponges are known for their colorful appearances and their morphological plasticity, encompassing encrusting, rope, ball and vase shapes ranging in size from a few mm to > 1m. Sponges are diploblast metazoans that lack true tissues or organs. In spite of their simple organization, genome sequencing has revealed genes encoding function that are highly homologus to those of their vertebrate analogs. As sessile filter feeders, they pump large volumes of water through a specialized canal system, termed the aquiferous system. The filtration capacities of sponges are remarkably efficient, leaving the expelled water essentially sterile. There are many bioactives isolated and screened various pharmacological activity. Many of them proved to be good drug molecules with significant results for preclinical and clinical studies. Some of which are discussed below: Marine sponges belonging to the genus Ircinia are known to be a very rich source of terpenoids, several of which have shown a wide variety of biological activities. Since terpenoids containing a tetronic acid moiety showed strong antibiotic activity. Eg: Variabilins, which were polyprenyl – hydroquinones, had analgesic and

anti-inflammatory properties. Among the halogenated alkaloids, bromoalkaloids form the most widely distributed group of natural compounds, which are predominantly found in marine eukaryotes like sponges, are significantly rarer in prokaryotic micro plants and animals.12 Components of marine sponges are known to modulate various biological activities and have antiinflammatory, anti fungal and anticancer effects. These in vitro activities imply that marine products may be potential therapeutic agents. Polyacetylenenic alcohols, including (35,145)-petrocortyre A, purified from the marine sponge Petrosia Sp., are biologically active lipid compound having similar structure to a long carbon chain compounds such as sphingolipids possess cytotoxic activity against a small panel of human solid tumour cell liner by inhibiting DNA replication.13 The high biological activity of Aplysina cavernicola, a much studied sponge which produces aeroplysinin and aerthionin and other dibromo and dichlorotyrosine derivatives, with some antibiotic activity against Bacillus subtilis and Proteus vulgaris. The sponge Ircinia ramosa has also been shown to possess antiviral, CNS stimulatory and antialgal properties.14 Red Sea Sponges has shown hypoglycemic effect in normal mice. An ethanol extract of Haliclona virdis showed a significant hypoglycemic effect lasting for more than 8 hr. after single oral doses of 200 or 500 mg /kg to normal mice.15 TUNICATES The Urochordata, sometimes known as the Tunicata, are commonly known as "sea squirts". 16 They are all sessile as adults. The name Tunicates arises from the existence of the tunic.17 Typically this tunic is attached to the substrate by a small holdfast and stands upright. It has two openings, an inhalant siphon and an exhalent siphon. The blood of tunicates is normally clear and often contains extremely high quantities of vanadium, a rare element normally occurring in very small quantities in sea water. Nobody yet seems to know why it should collect this vanadium.Tunicates are mostly hermaphroditic, meaning they are both male and female at the same time. Generally they avoid self fertilisation by either having the eggs and sperm chemically designed to reject each other, or by having the eggs and sperm mature at different times. Sperm are released into the sea but the eggs are retained within the body where they are fertilised by sperm brought in with incoming water. The eggs are brooded within the body until they hatch.18,19 Many of them are known to be a rich source of chemically diversity secondary metabolites with often remarkable biological activities. In many cases these compound are simple amino acid derivatives or more complex alkaloids. They often exhibit potent anticancer activities, so they are considered unusal cytotoxic metabolites. Perhaps, this property has limited the antimalarial potential of the pyridoacridones,

isolated from Cystodytes dellechiajei, and of bistramides, isolated from Lissoclinum bistreatum, as they possessed very narrow therapeutic indices.20 Iejimalides obtained from a marine tunicate Eudistomacf Rigida are unique 224-membered polyene macrolides having two methoxy groxy, four dienes units, and an N-formyl-L-serine terminus, and exhibit potent cytotoxic activity in vitro.21 Aromatic alkaloids possessing polysulfide structures have been isolated from ascidians of the genera Lissoclinum, Eudistoma and Polycitor. These compounds have shown various biological activities like antifungal, antibacterial, cytotoxicity, antimalarial activity, inhibition of protein kinase C. Three new active polysulfide aromatic alkaloids are found namely lissoclibadins 1,2,3, together with two known dimeric alkaloids, lissodinotoxins E and F.22 Halocidin is an antimicrobial peptide isolated from the hemolytes of the tunicate. Among the several known synthetic halocidin analogues, di-19HC has been previously confirmed to have the most profound antibacterial activity against antibiotic – resistant bacteria. This peptide has been considered to be an effective candidate for the development a new type of antibiotic.23 SEAWEEDS The term seaweed refers to the large marine algae that grow almost exclusively in the shallow waters at the edge of the world's oceans. They provide home and food for many different sea animals, lend beauty to the underwater landscape, and are directly valuable to man as a food and industrial raw material. Seaweeds are plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water. They are simpler than the land plants mainly because they absorb the nutrients that they require from the surrounding water and have no need for roots or complex conducting tissues. 24 Many seaweeds have hollow, gas-filled structures called floats or pneumatocysts. These help to keep the photosynthetic structures of the seaweed buoyant so they are able to absorb energy from the sun. The term thallus refers to the entire plant body of a seaweed. Seaweed draws an extraordinary wealth of mineral elements from the sea which includes sodium, calcium, magnesium, potassium, chlorine, sulfur and phosphorus; the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. It also contains several vitamins like carotenes (provitamin A); vitamin C, B12 along with higher proportion of essential fatty acids than land plants. Seaweeds provide a rich source of structurally diverse secondary metabolites which includes terpenes, acetogenins, alkaloids and polyphenolics, with many of these compounds being halogenated. The functions of these secondary metabolites are defense against herbivores, fouling organisms and pathogens; they also play a role in reproduction, protection from UV

radiation and as allelopathic agents. Chemical defense mechanisms that inhibit bioflim development are a common occurrence in seaweeds, with many secondary metabolites produced by seaweeds having bacteriocidal or bacteriostatic properties. Physical stress such as desiccation, UV and visible light and nutrient availability are able to alter the secondary metabolites in seaweeds.26,27 Some of the active algal specimens are Laminaria angustata var langissima, L.japonica, L.Japonica var. Ochotencs, Ecklonia cava and Esienia bicyclis and the green seaweed Monostrome nitidum. 28 The number and diversity of studies related to toxicity of marine algae are high. The first report on toxicity research are those of Doty and Anguilar-Santos and Aguilar-Santos and Doty, where the biological activity of the compound caulerpicine, isolated from caulerpa species was found to be toxic to mice. Norris and Fenical (1982) suggest that natural compound with biological activity are unusual or unique, generally halogenated or non-hologenated terpenoids synthesized by marine seaweeds alga to high herbivore pressure.29 The red alga Sphaerococcus coronopifolius was shown to have antibacterial activity; the green alga Ulva lactuca was shown to posses an anti-inflammatony compounds and an anti-tumor compound was isolated from Portieria hornemanii, Ulva fasciata produces a novel sphingosine derivative has been found to have antiviral activity in vivo. A cytotoxic metabolite, Stypoldione, which inhibits microtubule polymerization and thereby presents miotic spindle formation, has been isolated from tropical brown alga, Stypodium zonale. P.Hornemannii is found to be a novel source of cytotoxic penta halogenated monoterpene, halomon, which exhibited one of the most extreme of differential cytotoxicity in the screening conducted by the National Cancer Institute (NCI), USA. Haloman has been selected for preclinical drug development since this compound shows toxicity to brain, renal and colon tumor cell liner and preliminary in vivo evaluations have been encouraging. An iodinated novel nucleoside has been isolated from Hyprea volitiae, which is a potent and specific inhibitor of Adenosine Kinase.30 Crude Polysaccharide and Proteins from Himanthalia elongate and Cedium tomentosum have shown reduction in blood glucose after intravenous administration by 50% and 30% respectively at 5mg /kg dose.

SECONDARY METABOLITES FROM MARINE MICROORGANISMS

Biodiscovery and bioactive compounds The marine environment is emerging as a ‘gold mine’ for novel bioactive compounds with a staggering 1011 new compounds reported for 2009.

Marine-derived natural products present an enormous range of novel chemical structures and provide an interesting and challenging blueprint for creating new entities via synthetic chemistry. Marine invertebrates and plants, in particular, represent an environment rich in microorganisms that produce compounds with bioactive properties including antibacterial, antifungal, antiviral, anticancer, antifouling and antibiofilm activities. However, only 1% of these microorganisms can be isolated using traditional culturing techniques, which has been a major bottleneck when mining the marine environment for novel bioactive molecules.

Antimicrobial and antifungal The emergence of multidrug resistant bacteria and fungi, the latter including certain Aspergillus fumigatus and Candida albicans strains, drives the continuous search for novel antibacterial and antifungal agents. Most natural antibiotics used today originate from soil actinomycetes. However, since the rate of discovery of novel antibiotics of terrestrial origin is declining, other ecological niches, including the marine environment, are being exploited in the search for new antibiotics. Classes of compounds with antibacterial and/or antifungal activity which have been isolated from the marine environment include peptides, sterols, terpenes, alkaloids, and polyketides. Three classes of antibiotic resistant bacterial pathogens are emerging as major threats to public health: (i) methicillin-resistant Staphylococcus aureus (MRSA), (ii) multidrug resistant Gram negative bacteria, including Escherichia coli and Pseudomonas aeruginosa, and (iii) multidrug resistant Mycobacterium tuberculosis. Numerous compounds which could potentially combat these classes of pathogen have been isolated from the marine environment. These include structurally novel compounds such as marinopyrrole A and abyssomicin C with activity against MRSA, the alkaloid cyclostellettamine F with activity against P. aeruginosa, and trichoderins, novel aminolipopeptides with anti-mycobacterial activity. Compared with infections caused by drug resistant bacteria, infections caused by resistant fungal pathogens occur relatively infrequently. However, Candida species are a common cause of hospitalacquired bloodstream infection and kill 40% of those patients, whereas disseminated Aspergillus infections can kill up to 80% of affected patients. Compounds of marine origin with activity against these fungal pathogens include the cyclic depsipeptide kahalalide F and the alkaloid araguspongin C. 2.3.2.2 Antiviral Viral diseases, such as HIV and influenza A subtype H1N1, are a major threat to human health. Since viruses can rapidly evolve and develop resistance to currently used antiviral agents, discovering new antiviral drugs is of paramount importance. Many classes of antiviral compounds have been isolated from the marine

environment, including nucleosides, terpenes, cyclic depsipeptides, alkaloids, macrolides, and polysaccharides. The first commercial antiviral drug, Ara-A, was synthesised based on the structure of the nucleosides spongothymidine and spongouridine which were isolated from marine sponges. Although Ara-A and other synthetic nucleosides have been used to treat herpes simplex virus (HSV) and HIV, few marine antiviral compounds have entered preclinical trials. Examples of such compounds include the anti-HIV avarol, isolated from the marine sponge Disidea avara, and Cyanovirin-N, isolated from the cyanobacterium Nostoc ellipsosporum. 2.3.2.3 Anticancer Molecules with anticancer properties comprise the majority of bioactive molecules derived from marine sources. However, relatively few compounds enter preclinical and clinical trials and only a small group stems from microbes. Although chemically synthesised, araC (cytarabine) was designed based on the nucleosides from spongothymidine and spongouridine, originally isolated from the marine sponge, Tethya crypta, and is in clinical use for more than 40 years. Today, Yondelis®, a potent anticancer drug developed by Pharmamar from compounds produced by the tunicate Ecteinascidia turbinata, is probably the most successful example of how marine natural products can lead to anticancer treatments. 2.3.2.4 Antifouling and antibiofilm properties Biofouling, the undesirable accumulation of microorganisms, plants, algae, and/or animals on wetted structures, is of great concern in a wide range of applications, ranging from food packaging/storage, water purification systems, marine and industrial equipment, to medical devices. The two main strategies that are used to combat biofouling are to either prevent initial attachment or to degrade fouling biofilms. Several coatings are designed to prevent this initial attachment. However, some applications of the antifouling coating require certain requirements/restrictions. For example, many countries have now imposed a ban on the most effective antifouling coating (organotins) available for marine applications, urging instead the use of non-toxic novel biofouling compounds. In the health care sector, antifouling coatings for medical applications require compounds that are bactericidal and non-toxic to the human body. Marine organisms, in particular seaweeds and marine invertebrates, have proven to be a successful source of antifouling compounds. However, these marine compounds are difficult to obtain in large quantities. To overcome this problem, marine microorganisms are being explored to identify novel biofouling molecules which can be produced in larger quantities. Although the mechanism is unknown, several fatty acids (e.g. 1-hydroxymyristic acid, 9-Z-oleic acid and 12-methylmyristic acid) produced by marine

microorganisms have antifouling properties. The most promising evidence came from an experiment in which a coating consisting of 10% fatty acids prevented attachment of micro- and macro-fouling organisms on a panel which had been immersed in the ocean for 1.5 years. Similar results were obtained from a coating containing synthesised alkyl butenolide, after alkylated butenolides isolated from a deep sea Streptomyces species were found to exhibit antifouling properties. Several other compounds show promise including pyolipic acid, phenazine-1-carboxylic acid and 2-alkylquinol-4-ones and proteases, but their further potential is yet to be examined. Bacteria possess a cell-to-cell communication system termed quorum sensing. This communication system is dependent on the production of small molecules, which when sensed in high concentrations lead to coordinated behaviour by regulating several physiological processes including bioluminescence, motility, antibiotic resistance, virulence factor production and biofilm formation. Biofilms (aggregates of microorganisms where cells adhere to each other or to a surface) are of major concern for treating bacterial infections, since bacteria present in biofilms have increased antibiotic resistance profiles compared to their sessile counterparts. Since quorum sensing and biofilm formation are closely linked, it is not surprising that quorum sensing inhibitors often also exhibit antifouling/antibiofilm properties.

Recently, extracts from several microbial isolates from a marine habitat were shown to contain antiquorum sensing and antibiofilm activity against Pseudomonas aeruginosa, the primary cause of morbidity and mortality among cystic fibrosis patients. As such, quorum sensing and biofilm inhibitors could provide novel treatment options for treating bacterial infections, and these are urgently needed due to the emergence of multidrug resistant microorganisms.

ENDOPHYTIC FUNGI

Endophytic fungi Endophytes are microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects (Bacon 2000). As almost all vascular plant species appear to be inhabited by endophytic bacteria or fungi, these represent important components of microbial diversity. The relationship between the host plant and its endophyte shows symbiotic characteristics as the endophytic occupant usually obtains nutritients and protection from the host plant and in return profoundly enhances the fitness of the host by producing certain functional metabolites (Tan and Zou 2001). Still, if the host plant is weakened, the endophyte can also become an aggressive saprophyte and thereby reveal the smooth transition between symbiont and

opportunistic pathogen (Schulz and Boyle 2005). Fungal endophytes are a polyphyletic group of primarily ascomycetous fungi, whereas basidiomycetes, deuteromycetes and oomycetes are rarely found (Saikonnen et al. 1998, Arnold 2007). Although they do not show host specifity, certain fungal lineages appear with greater frequency in plants representing particular families and thus denote host preference (Cannon and Simmons 2002, Arnold 2007). Consistent with the tremendous diversity of endophytic fungi and their ecological roles is the astounding chemical variety of their secondary metabolites, which often display promising pharmaceutically or agrochemically exploitable activities when tested in various bioassays (Strobel et al. 2004). Due to the world's urgent need for new antibiotics, chemotherapeutic agents and agrochemicals to cope with the growing medicinal and environmental problems facing mankind, growing interest is taken into the research on the chemistry of endophytic fungi. Whereas between 1987 and 2000 approximately 140 new natural products were isolated from endophytic fungi (Tan and Zou 2001), a similar number was subsequently characterised in half of this time span, i.e. between 2000 and 2006 (Zhang et al. 2006). Many of these exhibit interesting activity profiles. Cryptocin (15), for example, is an tetramic acid isolated from the endophytic fungus Cryptosporiopsis quercina, an endophyte of Tripterigeum wilfordii, that possesses potent activity against the world's worst plant pests Pyricularia oryzae and other plant pathogenic fungi, advocating it for possible agrochemical usage (Li et al. 2000). From the medicinal plant Erythrina crista-galli the endophyte Phomopsis sp. was isolated, which produced the anti-inflammatoriy as well as antifungally and antibacterially active polyketide lactone, phomol (16) (Weber et al. 2004).

But it is not only new compounds being isolated from endophytes that are promising. The well known plant metabolite taxol (17), the "world's first billion-dollar anticancer compound" (Strobel 2004), was originally isolated from the bark of the endemic Pacific yew tree, Taxus brevifolia. It interferes with the normal function of microtubule breakdown. Specifically, taxol binds to the β-subunit of tubulin and thereby interrupts the dynamic rearrangement of this important component of the cytoskeleton. This adversely affects cell function because the shortening and lengthening of microtubules is necessary for their function as a mechanism to transport other cellular components, e. g. during mitosis. Thus taxol affects dividing cells, especially fast dividing ones like cancer cells. For the treatment of one patient suffering from cancer, 2 g taxol are required, which represents an amount equivalent to twelve trees and thereby posing a challenge to the limited natural resources, since the isolation from the inner bark implies

the destruction of yew trees. Thus, the demand for taxol greatly exceeds the supply that can be sustained by isolation from its natural source and alternative sources of the drug have been sought for a long time. Although the highly functionalized, polycyclic diterpene has been prepared by total synthesis, the process is too complex and not economically feasible. Currently, the supply of the compound is achieved by a successfully implied partial synthetic route based on baccatin III or its 10-deacetyl congener, which are isolated from the needles of other Taxus species and thus from a renewable resource. However, the extraction process of these precursors is tedious and costly. In the ongoing search for alternative sources of taxol, the group of Gary Strobel discovered taxol production in a hitherto undescribed endophytic fungus associated with Taxus brevifolia, identified as Taxomyces andreanae (Stierle et al. 1993). Although initially controversial, these findings prompted further studies, and it is nowadays an emerging picture that the ability to produce taxol upon fermentation seems to be a rather widespread feature among endophytic fungi. So far, more than 10 different fungal strains from at least 6 different host plants, most of them only distantly (if at all) related to Taxus, have been identified. However, it is worth mentioning that in all cases the resulting yields are minuscule, so far preventing any commercial exploitation (Strobel et al. 2004). Similar to the taxol case, the endophytic fungus Entrophospora sp. associated with Nothapodytes foetida was found to produce the cytotoxic plant alkaloid camptothecin (18) (Puri et al. 2005). The substance which was first described from the Chinese medicinal plant Camptotheca acuminata in 1966, exhibits remarkable anticancer activity by inhibition of the DNA enzyme topoisomerase I. Due to its low solubility and adverse drug reaction it is not used as an anticancer drug itself, but served as a drug lead and precursor for the semi-synthetic antiproliferatic drugs topotecan and irinotecan. Optimization of the fermentation conditions of the endophytic fungus may lead to the development of an economically and eco-friendly process for the production of camptothecin that could overcome the ever demanding supply problem (Puri et al. 2005) Thus, the ability to produce pharmacologically important natural products previously only known from plant sources is occasionally also inherent to endophytic fungi, whith further examples including podophyllotoxin (Puri et al. 2006, Kour et al. 2008) which will undoubtedly prompt future research into endophytic fungi.

Mangrove forests represent an ecosystem of high biodiversity (Kathiresan and Bingham 2001). It has been stated previously that biological diversity would imply chemical diversity, because the constant evolutionary race to survive would be most active (Strobel *et al*. 2004). Thus, in addition to their extraordinary morphological and physiological adaptations, the production of bioactive secondary metabolites might play an important role in the constant competition of mangroves with other plants, animals and microorganisms for the limited resources in their habitat.

In fact, the capability of mangroves to produce a wide array of bioactive compounds is reflected in numerous publications which describe the high chemical diversity of their metabolites, despite the fact that intensive research on mangrove metabolites only sprung up in the last two decades (Li *et al*. 2008). Several endophytic fungi have been isolated and cultured from the mangrove plants, *Rhizophora apiculata* and *Dendrophthoe falcate* (Kumaresan *et al*., 2002). More than 200 species of endophytic fungi have been isolated and identified from mangroves and have, despite the short period of research on the chemistry of mangrove endophytes, already been proven to be a well-established source for structurally diverse and biologically active secondary metabolites (Li *et al*. 2009).

Mangrove-derived fungi so far are still poorly investigated and thus represent a promising source of chemically new compounds with huge pharmaceutical and agrochemical potential. Hence, the present study is to investigate the production of enzymes and bioactive metabolites by endophytic fungi derived from the mangroves of Vellar estuary, Southeast India.

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PROBIOTICS

Definition of Probiotics

- **Better definition**: a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by insuring improved use of feed or by enhancing its nutrition, by enhancing the host response towards disease, or by improving quality of the ambient environment
- **Our focus**: response towards disease and improvement of the ambient environment
- Jobs of Microbial Adjuncts:
  - 1) microbial adjuncts preventing proliferation of pathogens in gut or elsewhere;
  - 2) improved digestibility;
  - 3) deliver improved nutrition to aquatics;
  - 4) enhancing host response to disease (acquired);
  - 5) improved environmental quality.

Possible Modes of Action

☐ production of inhibitory compounds

☐ competition for chemicals/available energy

☐ competition for adhesion sites (exclusion)

☐ enhancement of the immune response

☐ improvement of water quality

☐ interaction with phytoplankton

☐ a source of macro- and micro-nutrients

8 enzymatic contribution to digestion

(1) production of inhibitory compounds
(2) Release of chemicals having a bactericidal or bacteriostatic effect
(3) ultimate result: competitive edge for nutrients/energy
(4) production sites: in host intestine, on its surface, or in culture medium
(5) products: antibiotics, bacteriocins, siderophores, lysozymes, proteases, hydrogen peroxide, organic acids (pH change)
(6) exact compound is seldom identified: hence, the term **"inhibitory"**
(7) *Lactobacillus sp.* produces bacteriocins (toxins)

(8) marine bacteria produce bacteriolytic enzymes against *V. parahaemolyticus*

(9) *Alteromonas sp.* produces monastatin, shown to be inhibitory against *Aeromonas hydrophila*

(10) inhibitory effects have been shown by probiotics against aquaculture pathogens

(2) Competition for Chemicals or Available Energy

- Explains how different microbial populations exist in same ecosystem
- it is likely that it occurs in the mammalian gut, but proof is lacking
- application of the principles of competition to natural situations is not easy
- microbial situation in ecosystems is usually controlled by heterotrophs competing for organic substrates as both carbon and energy sources
- if you know the factors affecting microbial composition of the microbiota, ***you can manipulate it***
- All microorganisms require iron for growth
- **siderophore:** low mw ferric ion-specific chelating agents
- dissolve precipitated Fe and make it available for microbial growth
- siderophores scavenge Fe and make it unavailable to other species
- this occurs at tissue level
- probiotics producing siderophores can outcompete pathogens for Fe, thus limiting pathogen growth
- works best with pathogens that also produce siderophores (e.g., *V. anguillarum*)

(3) Competition for Adhesion Sites

- Competition for gut adhesion sites would limit colonization
- adhesion to enteric mucus is necessary for bacteria to become established in fish intestines

this is probably the first probiotic effect

- adhesion can be specific (based on adhesin and receptor molecules) or non-specific (based on physiochemical factors)

- total probiotic effect is probably a mixture of site competition, production of inhibitory compounds and nutrient/energy competition

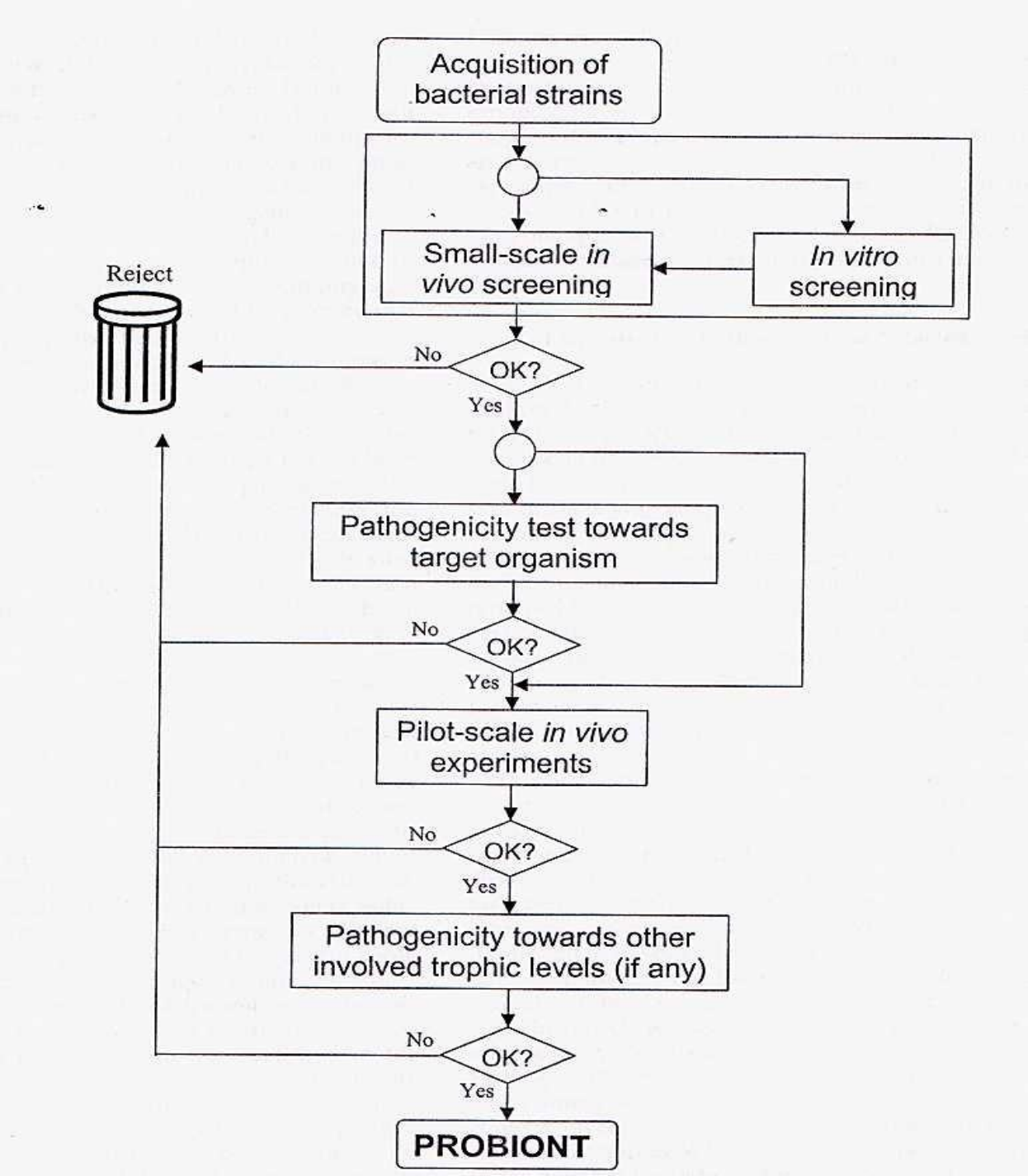
(4) Enhancement of Immune Response

- Rem definition of an immunostimulant? *Chemical compounds that activate the immune systems of animals and render them more resistant to infections by viruses, bacteria, fungi and parasites.*
- Immune response varies in animals

- lactic acid bacteria administered orally may induce increased resistance to enteric infections problem

(5) Improvement of Water Quality

- Proposed as a mode of action as a result of monitoring water quality after addition of probiotics
- usually associated with *Bacillus sp.*
- Hook: gram + bacteria are better converters of organic matter back to CO_2 than gram -
- thus: phytoplankton blooms are more easily maintained (interesting research area!)
- monitor: DOC, POC



Rationale for Selecting Probiotics

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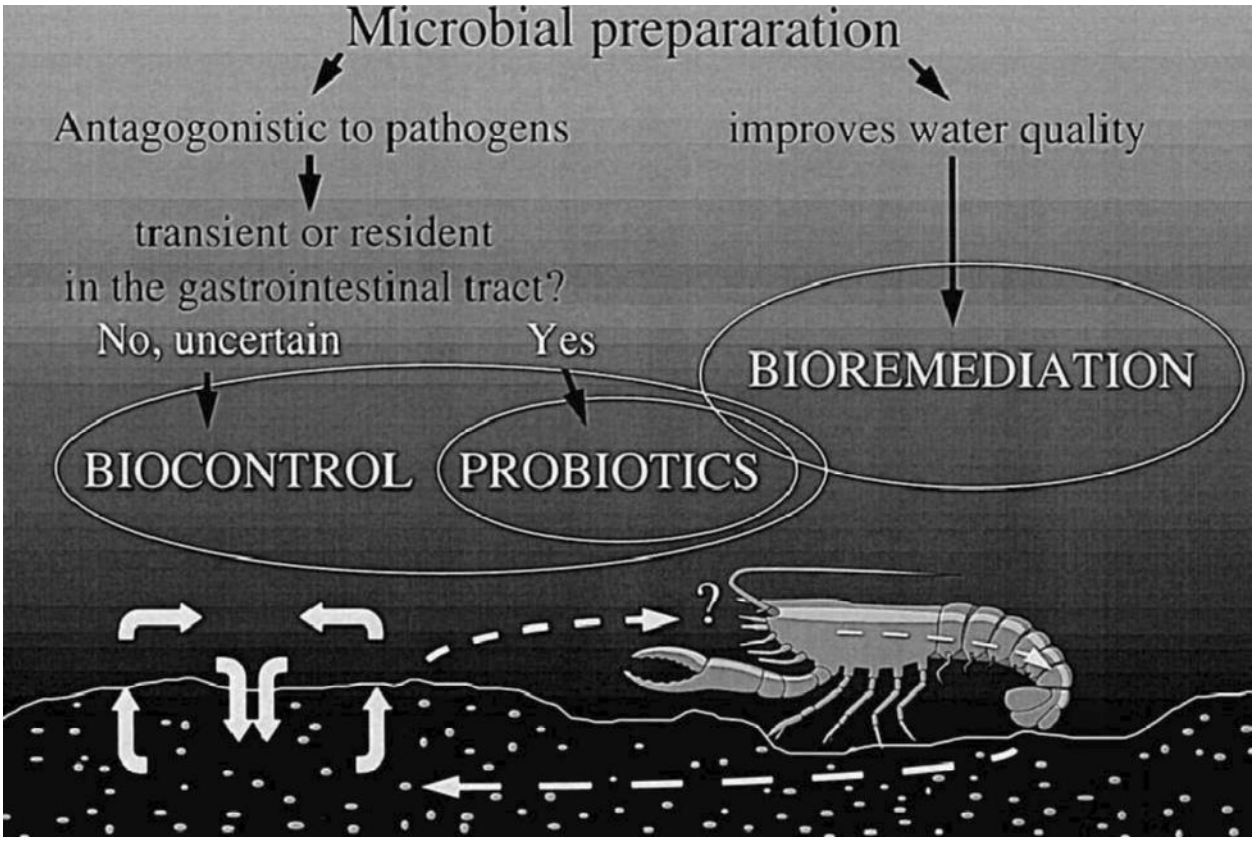
Microbial prepararation
Antagogonistic to pathogens
improves water quality
transient or resident
in the gastrointestinal tract?
No, uncertain
Yes
BIOREMEDIATION
BIOCONTROL
PROBIOTICS
?

KEY QUESTIONS

Unit –III

Part-A

1. What is meant by Biodiesel?
2. List out the uses of marine microalgae.
3. Comment on Marine endophytic fungi.
4. Write a note on Marine actinomycetes.
5. Define the term "Probiotics".

Part-B

1. Describe in detail the secondary metabolites produced by the marine organisms and explain their uses.
2. Write an informative account on marine cyanobacteria?
3. Write an elaborate account on marine actinomycetes?
4. Elaborately write about marine endophytic fungi?
5. Discuss the production of Probiotics for aquaculture?

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.Tech. BIOTECHNOLOGY

UNIT – IV - MARINE BIOTECHNOLOGY – SBT1304

UNIT-IV - Biomaterials and Bioprocessing - Marine byproducts: Fish oil, isinglass, fish glue, fish silage, fin rays, chitin, chitosan, agar, alginates, carrageenan and heparin.

FISH OIL

Fish oil can be obtained from eating fish or by taking supplements. Fish that are especially rich in the beneficial oils known as omega-3 fatty acids include mackerel, herring, tuna, salmon, cod liver, whale blubber, and seal blubber. Two of the most important omega-3 fatty acids contained in fish oil are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Make sure to see separate listings on EPA and DHA, as well as Cod Liver Oil, and Shark Liver Oil.
Fish oil is FDA approved to lower triglycerides levels, but it is also used for many other conditions. It is most often used for conditions related to the heart and blood system. Some people use fish oil to lower blood pressure, triglycerides and cholesterol levels. Fish oil has also been used for preventing heart disease or stroke, as well as for clogged arteries, chest pain, irregular heartbeat, bypass surgery, heart failure, rapid heartbeat, preventing blood clots, and high blood pressure after a heart transplant.

Fish oil is also used to for many kidney-related problems including kidney disease, kidney failure, and kidney complications related to diabetes, cirrhosis, Berger's disease(IgA nephropathy), heart transplantation, or using the drug called cyclosporine.
Fish may have earned its reputation as "brain food" because some people eat fish to help with depression, bipolar disorder, psychosis, attention deficit-hyperactivity disorder (ADHD), Alzheimer's disease, developmental coordination disorder, migraine headache, epilepsy, schizophrenia, post-traumatic stress disorder, and mental impairment.
Some people use fish oil for dry eyes, cataracts, glaucoma, and age-related macular degeneration (AMD), a very common condition in older people that can lead to serious sight problems.

Fish oil is taken by mouth for stomach ulcers caused by Helicobacter pylori (H. pylori), inflammatory bowel disease, pancreatitis, an inherited disorder called phenylketonuria, allergy to salicylate, Crohn's disease, Behcet's syndrome, and Raynaud's syndrome.

Women sometimes take fish oil to prevent painful periods; breast pain; and complications associated with pregnancy such as miscarriage (including that caused by a condition called antiphospholipid syndrome), high blood pressure late in pregnancy, early delivery, slow infant growth, and to promote infant development.

Fish oil is also taken by mouth for weight loss, exercise performance and muscle strength, muscle soreness after exercise, pneumonia, cancer, lung disease, seasonal allergies, chronic fatigue syndrome, and for preventing blood vessels from re-narrowing after surgery to widen them.

Fish oil is also used for diabetes, prediabetes, asthma, a movement and coordination disorder called dyspraxia, dyslexia, eczema, autism, obesity, weak bones (osteoporosis), rheumatoid arthritis (RA), osteoarthritis, psoriasis, an autoimmune disease called systemic lupus erythematosus (SLE), multiple sclerosis, HIV/AIDS, cystic fibrosis, gum disease, Lyme disease, sickle cell disease, and preventing weight loss caused by some cancer drugs.

Fish oil is used intravenously (by IV) for scaly and itchy skin (psoriasis), blood infection, cystic fibrosis, pressure ulcers, and rheumatoid arthritis (RA).

Fish oil is applied to the skin for psoriasis.

How does it work?

A lot of the benefit of fish oil seems to come from the omega-3 fatty acids that it contains. Interestingly, the body does not produce its own omega-3 fatty acids. Nor can the body make omega-3 fatty acids from omega-6 fatty acids, which are common in the Western diet. A lot of research has been done on EPA and DHA, two types of omega-3 acids that are often included in fish oil supplements.

Omega-3 fatty acids reduce pain and swelling. This may explain why fish oil is likely effective for psoriasis and dry eyes. These fatty acids also prevent the blood from clotting easily. This might explain why fish oil is helpful for some heart conditions.

****

Isinglass

Isinglass (/ˈaɪzɪŋglæs/ or /ˈaɪzɪŋglɑːs/) is a substance obtained from the dried swim bladders of fish. It is a form of collagen used mainly for the clarification or fining of beer. It can also be cooked into a paste for specialized gluing purposes.

Its origin is from the obsolete Dutch *huizenblaas* - *huizen* is a kind of sturgeon, and *blaas* is a bladder. [1]

Isinglass was originally made exclusively from sturgeon, especially beluga, until the 1795 invention by William Murdoch of a cheap substitute using cod. This was extensively used in Britain in place of Russian isinglass. The bladders, once removed from the fish, processed, and dried, are formed into various shapes for use.

Foods and drinks

Before the inexpensive production of gelatin and other competing products, isinglass was used in confectionery and desserts such as fruit jelly and *blancmange*.

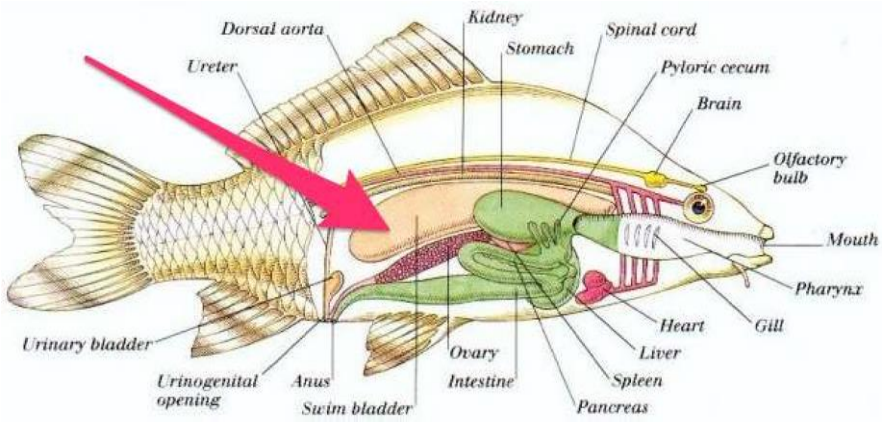
Isinglass finings are widely used as a processing aid in the British brewing industry to accelerate the fining, or clarification, of beer. They are used particularly in the production of cask-conditioned beers, although a few cask ales are available which are not fined using isinglass. The finings flocculate the live yeast in the beer into a jelly-like mass, which settles to the bottom of the cask. Left undisturbed, beer will clear naturally; the use of isinglass finings accelerates the process. Isinglass is sometimes used with an auxiliary fining, which further accelerates the process of sedimentation.

Non-cask beers that are destined for kegs, cans, or bottles are often pasteurized and filtered. The yeast in these beers tends to settle to the bottom of the storage tank naturally, so the sediment from these beers can often be filtered without using isinglass.[*citation needed*] However, some breweries still use isinglass finings for non-cask beers, especially when attempting to repair bad batches.

Although very little isinglass remains in the beer when it is drunk, many vegetarians[2] consider beers that are processed with these finings (such as most cask-conditioned ales in the UK[3]) to be unsuitable for vegetarian diets (although acceptable for pescetarians).[4] A beer-fining agent that is suitable for vegetarians is Irish moss, a type of red algae also known as carrageenan.[5] However, carrageenan-based products (used in both the boiling process and

after fermentation) primarily reduce hazes caused by proteins, but isinglass is used at the end of the brewing process, after fermentation, to remove yeast. Since the two fining agents act differently (on different haze-forming particles), they are not interchangeable, and some beers use both.

Isinglass finings are also used in the production of kosher wines, although for reasons of kashrut, they are not derived from the b ϵluga sturgeon, as this fish is not kosher.[6]Wh ther the use of a nonkosher isinglass renders a beverage nonkosher is a matter of debate in Jewish law. Rabbi Yehezkel Landau, in *Noda B'Yehuda*, first edition, Jore Deah 26, for example, permits such beverages.[6] This is the position followed by many kashrut-observant Jews today.



FISH GLUE

Fish glue is often made by heating the skin or bones of fish in water. It can also be made from part of the fish's air bladder which, in the case of glue made from sturgeon, is called isinglass. Adhesives made from fish, as well as hide glue made from other animals, were sometimes used in ancient Egypt. They are still used in art, for shoe and furniture repair, and to preserve old manuscripts. Hide glue is typically manufactured from the skin of non-oily fish.

Fischleim, flüssig
Fish glue, liquid

During medieval times in Europe, fish glue was often used to repair animal-based sheets called parchments, which were used for writing. It was also used in painting materials by some artists in China. Paintings and drawings were often coated with this type of glue in the 1800s. While the glue by itself is typically brittle, it can be used along with other materials to restore paintings.

Artistic uses for the glue include its application as a binder, glazing agent, or protective coating for paintings. The substance can also be used for building and repairing wooden antiques, as well as building new products. It doesn't always hold a piece enough if gravity is pushing on it, so fish glue is sometimes used in combination with other types of glue when securing objects. Some manufacturers produce such glues that can last in a bottle for a couple of years before being used.

Adhesives made from animal glue are often made out of the collagen in skin and other tissues. In addition to fish, a number of animals such as rabbits and horses have been used to make glue. While glue factories were built in places like Holland and the United States, fish glue is not typically found as an industrial product. Many products, however, include a similar compound called gelatin, including deserts, marshmallows, and capsules for pharmaceutical pills. Fish glue is often sensitive to changes in humidity and temperature, and can shrink while drying, so it is not always the preferred medium to use.

People who work as artists or in wood shops sometimes use fish glue for certain projects. For most other applications, synthetic glues that can withstand many harsh conditions are generally used. These glues are sometimes applied because these tend to stick different materials together and are typically flexible. The adhesives, however, often need to be processed in heated pots or have chemicals added to remain a liquid where used.

What is Fish silage?

Fish silage as described here is defined as a liquid product made from whole fish or parts of fish that are liquefied by the action of enzymes in the fish in the presence of an added acid. The enzymes break down fish proteins into smaller soluble units, and the acid helps to speed up their activity while preventing bacterial spoilage.

Silage made from white fish offal does not contain much oil, but when it is made from fatty fish like herring it may be necessary to remove the oil at some stage.

There are other methods of making liquid fish protein, for example by adding enzymes or bacteria, but these are not described here.

How is fish silage made?

The raw material is first minced; suitably small particles can be obtained by using a hammer mill grinder fitted with a screen containing 10 mm diameter holes. Immediately after mincing, 3·5 per cent by weight of 85 per cent formic acid is added, that is 35 kg or about 30 litres of acid to one tonne of fish. It is important to mix thoroughly so that all the fish comes into contact with acid, because pockets of untreated material will putrefy. The acidity of the mixture must be pH 4 or

lower to prevent bacterial action. After the initial mixing, the silage process starts naturally, but occasional stirring helps to ensure uniformity.

The production tank can be of any size or shape provided it is acid resistant; some steel containers used for making or carrying the silage may need a polyethylene liner to prevent corrosion. Concrete tanks treated with bitumen are suitable for holding large quantities. The size and number of tanks depend on the amount and type of raw material available.

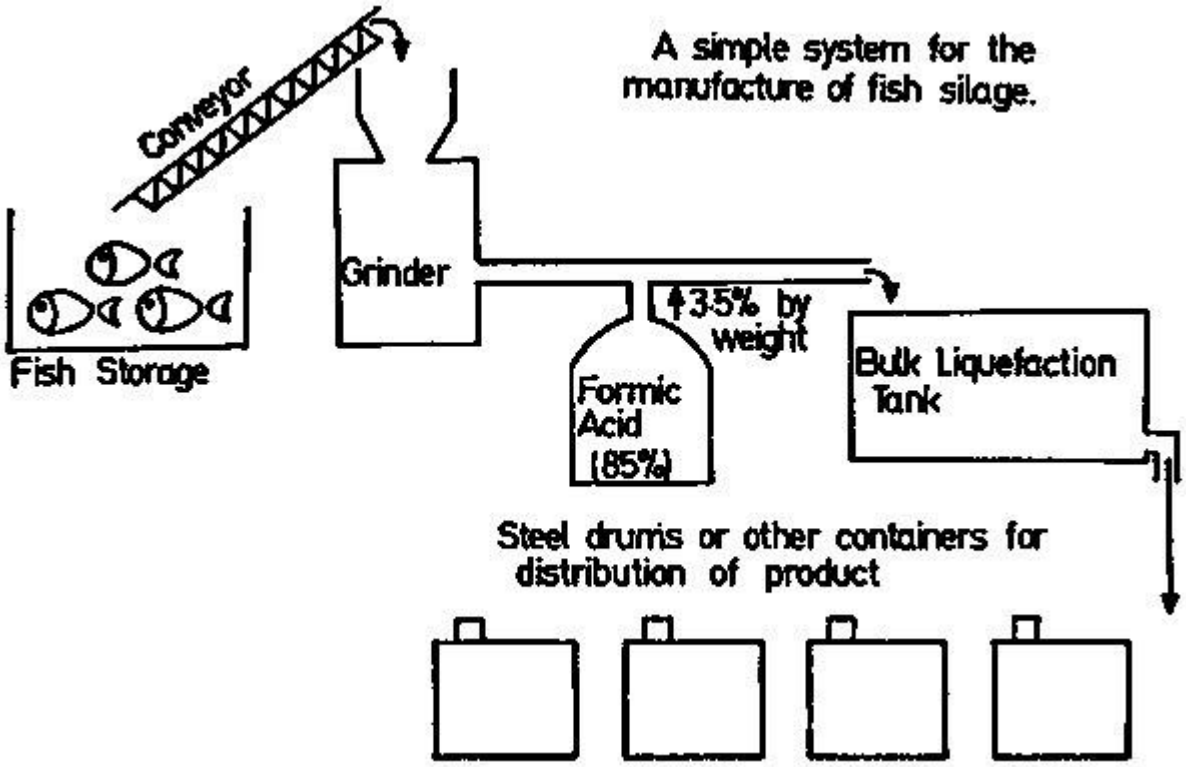
The rate of liquefaction depends on the type of raw material, its freshness, and the temperature of the process. Most species can be used, but sharks and rays are rather difficult to liquefy, and should be mixed in with other species. Fatty fish liquefy more quickly than white fish offal, and fresh fish liquefy much more quickly than stale fish. It should be possible in most installations to mince and add the acid immediately the raw material is received, thus avoiding slow liquefaction of stale fish. The warmer the mixture, the faster the process; silage made from fresh white fish offal takes about two days to liquefy at 20°C, but takes 5-10 days at 10°C, and much longer at lower temperatures. Thus in winter it would be necessary to heat the mixture initially, or to keep it in a warm area until liquid.

Minced untreated fish must be kept covered to keep out flies; once the acid has been added, flies are not attracted to the mixture.

Once the silage is prepared it can be handled like any other liquid, and transported in bulk or in containers. It can also be blended with cereals to make a semidry feed. Silage made from white fish offal should be stirred as it is removed from the production tank to obtain a uniform batch, since a bone-rich layer tends to settle at the bottom of the tank after a time. Silage made from fatty fish is more homogeneous and there is little separation even after prolonged storage, but the oil in it deteriorates very rapidly; if the oil has to be removed and used for other purposes, it can be separated by heating and centrifuging.

Fish silage can be concentrated to reduce its bulk, but more experimental work needs to be done to assess the commercial advantage of such a process.

A simple system for the manufacture of fish silage.



What acids can be used?

Several acids can be used, either alone or in combination. Hydrochloric or sulphuric acid can be used; they are reasonably cheap, but a lower pH is required with these mineral acids than with some organic ones, and this means greater corrosion problems, and the silage has to be neutralized before use. Formic acid, an organic acid, is a good choice because preservation is achieved at a slightly higher pH, it has some bacteriostatic action, and the silage need not be neutralized before adding it to the feed, but it is more expensive than mineral acids.

Acids must be handled with care, and formic acid is no exception; operators should always wear rubber gloves and goggles. The acid storage tank, made of resistant material, should be inaccessible to unauthorized people.

The composition of fish silage

The composition of fish silage is very similar to that of the material from which it is made. A typical analysis of white fish offal is 80 per cent water, 15 per cent protein, 4·5 per cent ash and 0·5 per cent fat, and the composition of silage from offal is virtually the same. Whole fatty fish like sprats and sand eels have a higher protein and fat content, and correspondingly lower water and ash content.

Samples from a batch of silage for analysis should be taken only after thorough mixing to ensure that they are representative. Acidity should be measured when making large batches; with formic acid the pH should be 3·6-4; if it is above 4 more acid should be added; if it is below 3·8 less acid could probably have been used, with a saving in cost. The exact amount of acid has to be found by experience, but the proportion given earlier is a good guide.

How long does fish silage keep?

Fish silage of the correct acidity keeps at room temperature for at least two years without putrefaction. The protein becomes more soluble, and the amount of free fatty acid increases in any fish oil present during storage, but these changes are unlikely to be significant nutritionally. Fish silage in any event would probably not be stored commercially for more than about 6 months. Silage becomes smoother in consistency during storage, and develops a pleasant malty odour.

How is fish silage used?

Fish silage is used in the same way as fish meal in animal feed. Fish meal contains about 65 per cent protein whereas fish silage contains about 15 per cent, so that about four times as much silage is required for the same protein intake. The most suitable outlet for silage appears to be in pig farming, since it can be used in liquid feeding systems. Silage can be used alone, or with fish meal; feeding trials show that pigs grow as fast on silage as on meal, and the quality and flavour of the meat is good. Fish silage is used in the Danish pig industry, and most nutritional work has been done there. Other animals have been fed on silage with good results; cow's milk and butter are without taint, and egg production from hens is high.

The pros and cons of making silage

The main advantage of the fish silage process is that, in areas where there is no fish meal factory, fish offal and waste fish can be utilized instead of being thrown away.

The advantages and disadvantages of making fish silage instead of fish meal can be summarized as follows.

Capital cost of meal plant is fairly high; the cost of silage equipment is fairly low.

Processing of meal requires engineers and technical staff; silage can be made by unskilled workers.

Smell is a problem when making meal, unless specially equipped plant is used; there is no smell when making silage.

Transport of meal is cheap, because the stable concentrated powder is low in bulk; silage is more expensive to carry because the liquid, which contains all the water that was in the fish, is four or five times as bulky as meal.

Marketing of fish meal is long established and the product is well known; silage is little known in the UK and if anything more than local production and use is envisaged, some marketing effort would be required. Silage manufacture might sometimes serve as a preliminary step towards making fish meal by proving the existence of a sufficient supply of raw material before making a large investment.

Where might silage be the answer?

The silage process is most likely to be successful in areas where fish offal or waste fish is regularly available, but the cost of sending it to the nearest meal plant is prohibitive, and where there are farms, particularly pig farms, close by.

There is no limit to the size of a silage plant; a batch can be made in one oil drum or in a tank holding several tonnes. Each situation has to be considered on its own merits, but the larger the potential production and the greater the distance the silage has to be carried the more likely it is that meal manufacture is more appropriate.

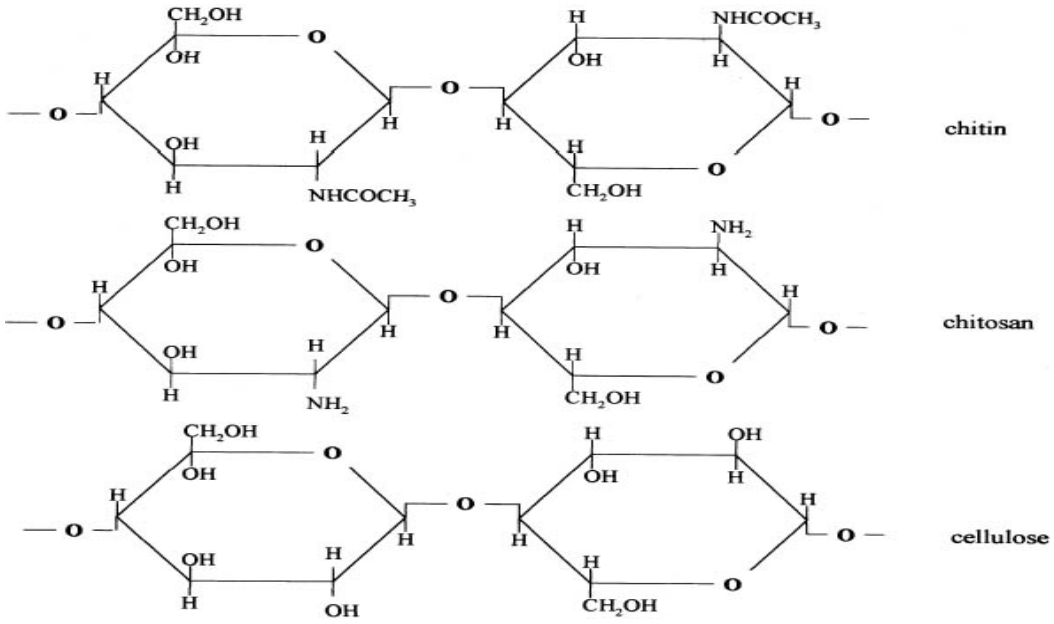
Shark fins (Fin Rays)

The commercial value of the fins depends on their color, size, variety and quality. Depending on the quality and quantity of rays present in the fins they are broadly classified into 2 verities, generally known as black and white. Black fins usually fetch a lower price than white fins. The translucent cartilaginous rods embedded in the fins of shark are the fin rays used in the preparation of shark fin soup. These rays can be extracted from both freshly cut as well as dried fins. The latter are soaked in water which is acidified with acetic acid with pH 2.5 to 5 for 2-3 days while freshly cut fins require less soaking time. The softened fins are then treated with hot 10% acetic acid at 60°C for an hour depending upon their size. The rays are separated manually, washed well and dried in the sun. The dried rays which can have a moisture level of 5-8% are stored in polyethylene bags. The shark fin soup is considered as a delicacy in countries like China, Philippines, Hongkong, Singapore, etc. shark fins are in great demand particularly among Chinese, for making ceremonial dish called shark fin soup.

CHITIN AND CHITOSAN

Chitin is a white, hard, inelastic, nitrogenous, polysaccharide found in the outer skeleton of insects, crabs, shrimps and lobsters and in the internal structures of invertebrates.

Chitosan is deacetylated chitin, and is polymer of ß (1-4) acetyl - D glucosamine.
It has multifarious uses in the cosmetic, pharmaceutical and medical industries.
It is even considered as a wonder drug of the twenty-first century due to its versatile utility.



Potential

Scope for chitin/ chitosan production in India

The international shrimp industry from harvest through various processing operations produces a vast amount of potentially recoverable proteinaceous by-products in the form of shrimp heads and shells which is one of the major raw materials for chitin/chitosan production.

Shells of other crustaceans viz. crabs, lobsters, squilla, cuttle fish bones also could be profitably utilised.

Squilla

In India, it is estimated that more than one lakh tons of shrimp processing waste is being wasted annually which could be gainfully utilised for manufacturing chitin a high value industrial product. Another raw material for chitin is squilla.

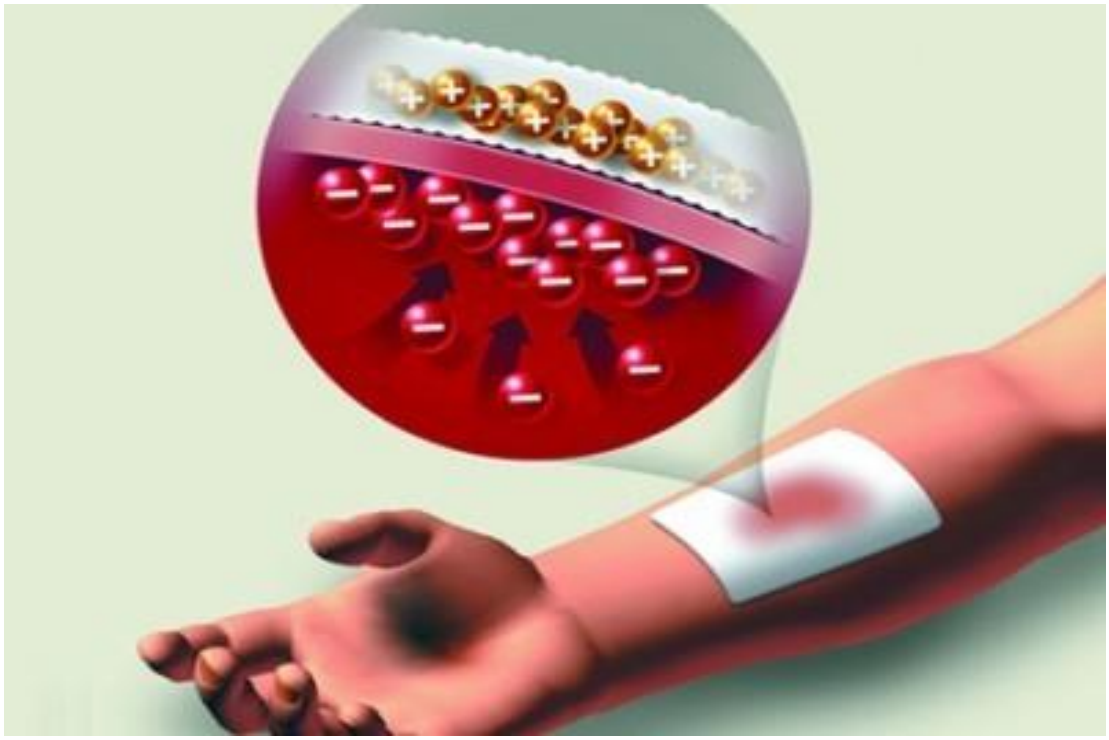
It is estimated that a potential of around 50,000 tons of squilla is available of which nearly 5,000 ton is being thrown back into the sea. This is an important trawl by catch especially in Mangalore and could be used for chitin/chitosan production.

Crab shells and lobster shells are also raw materials for chitin/chitosan production.

The estimated availability of crab shells is 30,000 - 40,000 tons in the Indian waters.

Important properties of chitosan

Medical grade micronised chitosan is biodegradable, non allergic, haemostatic, non toxic and wound healing accelerator. Chitosan films are flexible, tough, transparent, clear and oxygen permeable with good tensile strength. Chitosan could be used to make single and bipolymer membranes , non woven fabrics and sponges for surgical applications. It is resistant to alkali, digestive enzymes and urine. Chitosan also could be cross linked.



Uses of Chitosan

i) <u>Clarification and Purification</u>

- The property of long chain molecules of dissolved Chitosan to wrap the solid particles suspended in liquids and to bring them together and agglomerate makes it suitable as a coagulant aid.
- It is used in treatment of sewage effluents, purification of drinking water etc.

ii) Chromatography

The presence of free amino acid hydroxyl groups in chitosan is a good chromatographic support.

iii) Paper and Textiles

The high molecular weight, poly cationic linear film forming and hydrogen bonding ability makes chitosan an ideal polymer applicable in the paper industry.

The chelating ability, adhesive property and ionic bond forming characteristic of chitosan find potential application in textiles.

Fabric seized with chitosan have good stiffness, improved dye uptake, added lustre and improved laundering resistance.

iv) Photography

Due to the resistance of chitosan to abrasion, optical characteristic film forming ability and behaviour with silver complexes chitosan has important application in photography.

v) Food and Nutrition

Chitosan supplemented chick feed and fish feed improved the weight gain in chicken and fish.

vi) Agriculture

It has potential application in agriculture such as germination and culturing to enhance self protection against pathogenic organisms in plants and suppress them in soil, to induce chitinase activity, in encapsulation of fertilizers, in liquid fertilizers and in controlled release of herbicides.

vii) Medical and Pharmaceutical

- bacteriostatic agent
- drug delivery vehicle
- enzyme immobilization
- film / membrane for dialysis
- artificial skull sponge for mucosal haemostatic agent wound dressing
- anti cholesteremic material
- anti sore composition
- antibillirubinemia agent
- preparation of self regulated drug delivery system
- sustained release / direct compression matrix tablets.

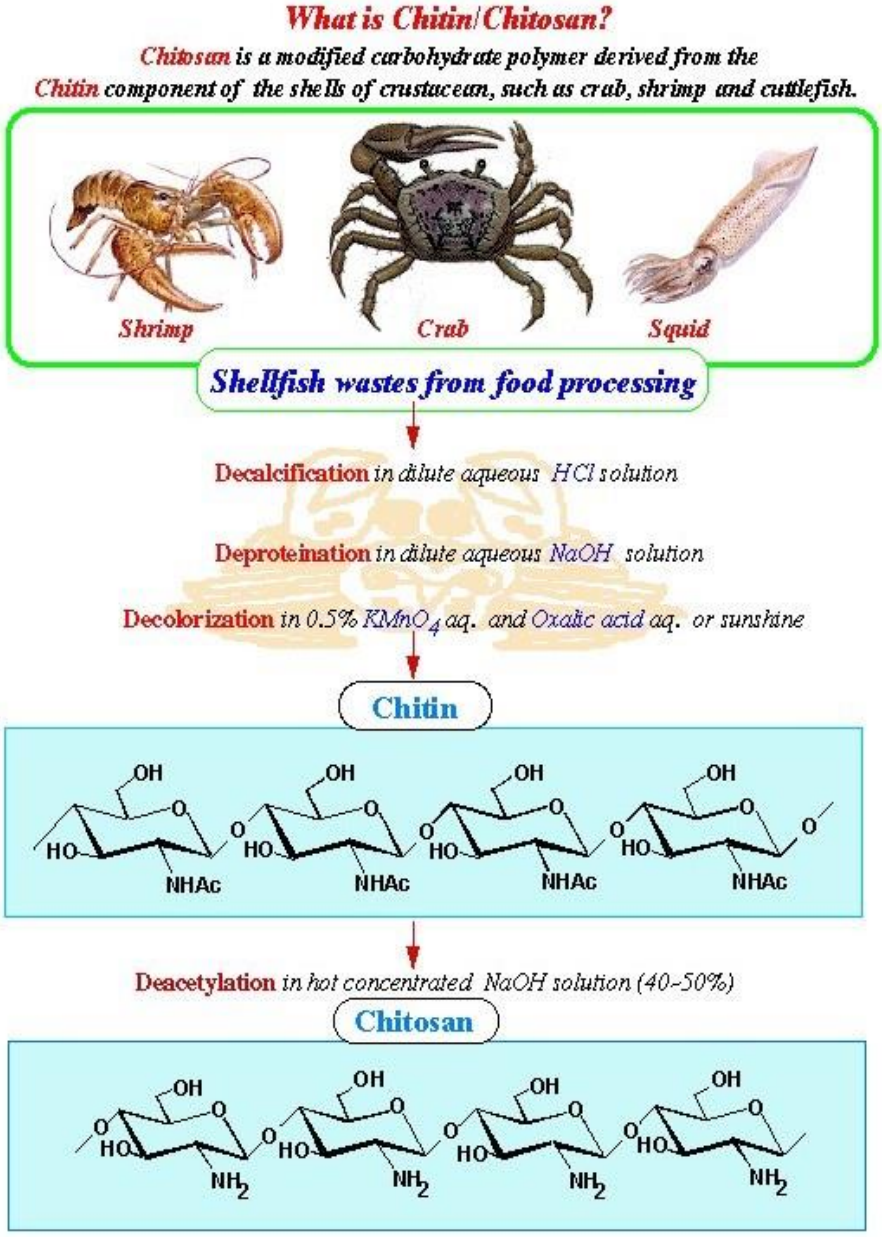


Fig. 2. Prepration of chitin and chitosan

Technology

- Central Institute of Fisheries Technology (CIFT) Kochi has the distinction of perfecting the technology for chitin and chitosan production in the country.
- The institute is imparting training to entrepreneurs who are interested in setting up such units.

Raw material

- Dried/wet shells of prawns, squilla, crabs, lobsters etc., could be utilised.
- The shells thus used should be thoroughly free from sand and extraneous matter, so as to reduce the ash content of the final product to less than 2%.

Deproteinisation

The shells are boiled with 3% NaOH for 30 minutes in a mild steel vessel to remove protein stuck to head and shell. The boiled raw material is allowed to cool and it is washed with water to remove all traces of alkali (could be tested with a pH paper).

Demineralisation

- The deproteinised shells are transferred to a mild steel vessel lined with fiber glass and is treated with 3% HCl.
- This is kept for 30 minutes with occasional stirring till the reaction is complete.
- The excess acid is decanted and the residue is washed till the pH is normal.

Removal of water

- Excess water is removed using a screw press till the moisture is below 60%.
- The product thus obtained is called chitin.

Deacetylation of Chitin

- It is the process of conversion of chitin to chitosan.
- Chitin is heated at 90-95ºC for about one and a half hour with 40% Na_2CO_3 (caustic soda) in a mild steel vessel.
- Excess alkali is drained off and the mixture is washed with water several times till it is free from alkali.
- 85% of the alkali, thus removed could be reused in subsequent cycles.

Removal of water

- Excess water is removed in a screw press and the product thus obtained is wet chitosan

Drying

The above product is sun dried for 6-8 hours or in drier till the moisture content is less than 5%. Care should be taken not to exceed the drier temperature beyond 60ºC. Chitosan thus obtained is in the form of flakes.

Powdering and Packing

The chitosan flakes obtained could be powdered and packed in lots of 10,20,25 kg in HDP/ Polyethylene lined non woven sacks in a dry place. Chitin can be stored for one year whereas Chitosan can be stored for nearly three months only.

Yield

Chitin represent 14-27% and 13-15% of the dry weight of shrimp and crab processing waste respectively and squilla yields 15% chitin. On a conservative basis the yield is estimated as

- Dry raw material chitin 14% by wt
- Chitosan 10% by wt.

- Thus from 1000 grams of dry shell, we get a yield of 140 grams of chitin and 100 grams of chitosan.

Uses

- Chitosan finds use as a sizing material for rayon and other synthetic fibres, cotton, wool etc.
- It may also be used in the preparation of cosmetics and Pharmaceuticals and also as a water-clarifying agent.
- Medical application of chitosan in the form of chitosan impregnated gauze and chitosan film
- for treatment of chronic wounds and external ulcers,
- to arrest/minimise bleeding in neurosurgery,
- as artificial skin and kidney membrane,
- in plastic surgery,
- as contact lens,
- in periodontal application etc.

****

Agar Production

INTRODUCTION

1. In addition to edible and other direct uses, marine algae provide a rich and diverse source of raw material for the manufacture of seaweed gums, a group of natural compounds characterized by their thickening and gelling properties.
2. Such compounds find wide application in the food, pharmaceutical and industrial sectors.

3. The three most important of these compounds, in terms of volume and value, are sodium alginate (and its derivatives), carrageenan and agar.
4. An estimate in 19801 put total world production of these three gums at around 40,000 tons, valued at US$300 million.
   This was obtained from 150,000 t (dry weight) of seaweed. A more recent estimate has put annual world production at:

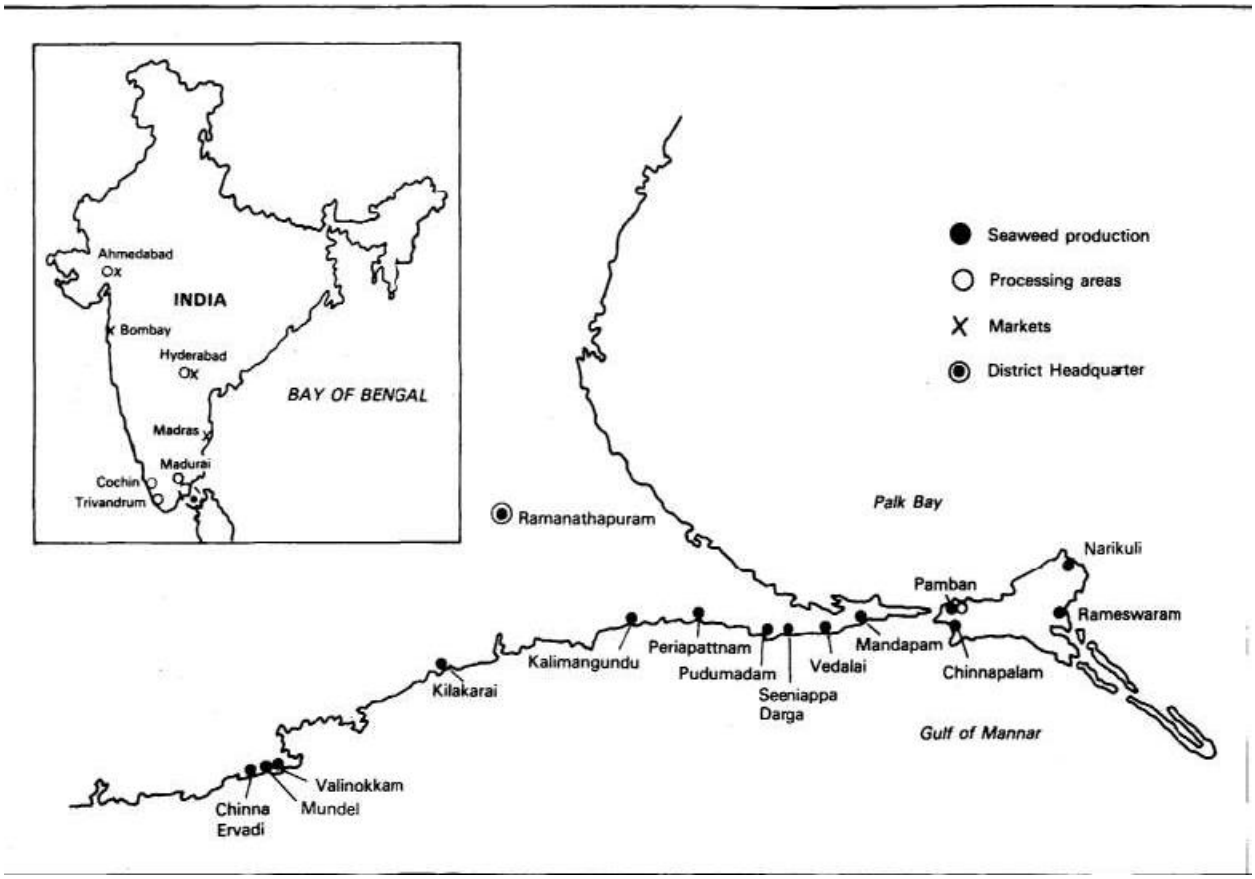
- Agar 7,000—10,000
- Carrageenan 12,000—15,000
- Alginates 22,000—25,000
-

PRODUCTION AND TRADE iN SEAWEED AND SEAWEED GUMS

- World production and trade in seaweed is very large, but the greater part of this is intended for edible use, mainly in Japan, China and South Korea.
- Agar is obtained from certain red seaweeds, the most important of which are from the genera *Gracilaria, Gelidium* and *Gelidiella.*
- Japan is the main consumer and producer of agar;
- Most of the 10,000 t of agar—yielding seaweed (agarophytes) that is estimated to enter international trade each year is imported by Japan.
- Of this world-wide harvest, 63% comes from Chile, with the Philippines (15%), South Africa (10%) and Brazil (6%) also significant suppliers.
- In 1984, Japanese production of agar amounted to almost 2,500 t (37% of the total world production of around 6,700 t);
- other important producers were Spain (13%), Chile (12%), South Korea (9%) and Morocco (8%).
- Alginates are obtained from a number of different brown seaweeds and since many of these are cold- or temperate-water types,
- The main alginate producers (USA, UK, Norway and France) are able to use indigenous sources of weed, such as *Laminaria, Macrocystis* and *Ascophyllum.*
- An exception is Japan, which relies on imports of weed from Norway.
- Over a third (8,000-10,000 t) of the world's alginate production is estimated to be used in developing countries, mainly in Asia, and almost all of it is imported by them.

India

- **current domestic production is around 75 tons/yr (estimated).**
- **This would represent 1% of the total world production in 1984.**
- **Total production is set to almost double to around 140 t/year within the next few years.**



Production and marketing areas for seaweed. agar and alginates

- By international standards, Indian agar and alginates do not compare favourably in terms of gel strength and viscosity.
- Although the Indian products are competitive on a cost basis and are not subject to export restrictions,
- the world market offers little scope for most categories of Indian produced agar and alginates.
- But occasional shipments of agar have been made by the larger producers to the USA, UK and Japan
- From 1975 to 1984 there was a ban on the export of seaweed from India.
- The present export policy of the Government of India (1988-91) permits export 'on merit' of all types of seaweed against the procurement of a licence.
- Such a licence is usually granted by the Joint Controller of Import and Export after due consideration of each application.
- The present import policy of the Government of India (1988-91) restricts the import of seaweed and other algae, fresh or dried, to licence holders who have to apply and fulfill conditions laid down under the Export Control *Act* of 1955

which is administered by the Central Government and the Chief Controller of Imports and Exports or an authorized official.

SEAWEED COLLECTION IN INDIA

- **Location**
- Although processing takes place in several states, commercial harvesting of seaweed, all from natural sources, is limited to the southern portion of the Tamil Nadu coastline, from Kanyakumari (Cape Comorin) in the south, northwards to the peninsula that forms the Gulf of Mannar, a total distance of almost 300 km.
- Collection is particularly concentrated in that part of the 'seaweed belt' that runs along the coast of Ramanathapuram District and includes the villages of Mundel, Valinokkam, Chinna Ervadi, Kilakarãi, Kalimangundu, Periapattnam, Pudumadam, Seeniappa Darga, Vedalai, Pamban, Chinnapalam and Rameswaram.
- Here, the seaweed is collected both from the waters
- off the mainland coast and those surrounding the chain of off-shore islands**.**

Species of seaweed utilized

- Agarophytes used in commercial processing are species of the genera *Gracilaria, Gelidium* and *Gelidiella.*
- Where the gel strength of the agar is not critical, as in food use, *Gracilaria* alone may be utilized.
- In other cases, and whenever bacteriological/IP grade agar is produced, *Gelidium* or *Gelidiella,* separately or mixed with *Gracilaria,* is utilized.
- (The terms *Gelidium* and *Gelidiella* are often used interchangeably within the industry although they are recognized as being separate genera).
- There is undoubtedly some variation in the distribution of the different seaweed varieties along the coast, but it is not possible to define this with any precision.
- *Gracilaria edulis* (syn. G. *lichenoides),* found along the whole of the Tamil Nadu coastline, is the most abundant and commonly used *Gracilaria* species; G. *verrucosa* (syn. G. *confervoides)* occurs in estuarine/brackish water areas, such as are found near Tuticorin, and is utilized by one or two producers.
- Other *Gracilaria* species found in Indian waters include G. *crassa, G. corticata* and G. *multipartita* (syn. G. *folizfera),* and any one, or more, of these may be present in indeterminate amounts in what is sold as *'Gracikiria'* or *G. edulis*
- The particular species of *Gelidium* used do not appear to be identified within the industry, either by the seaweed agent or the processor.
- *Gelidiella* is invariably stated to be *Gelidiella acerosa* and tends to be found in slightly more rocky areas than, for example, *Gracilaria.*

- Indian alginate production is derived from *Sargassum* and *Turbinaria* seaweed.
- Industry sources do not usually name the particular species used, but the species are believed to be predominantly *Sargassum wightii* and *Turbinaria conoides*, with some *S. myriocystum* and *T. ornata.*

Seasonality of collection

- Most seaweeds are generally available from agents throughout the year, although there are a few months when stocks are especially plentiful and others when they are less so.
- Processors and agents agree that there is a peak in *Gracilaria* collection during January-April.
- *Gelidium/Gelidiella* are usually described as being non-seasonal, although occasionally the reverse is acknowledged.
- It is probable that growth of the seaweed varies somewhat according to local conditions and this, together with other factors, such as prevailing weather conditions and the extent to which seaweed collection is a primary or secondary activity within the fishing community, gives rise to the 'seasonality' observed.
- There may be no collection for a short period around June, when the seas are rough, or in November, during the heavy rains. July-August, when high winds have torn the weeds free from their growth points, is said to be a peak period for *Sargassum* collection.

Methods employed

- For the people of the coastal villages, fishing is their main income and seaweed collection is an important second source of income;
- for the women who are otherwise not actively employed in fishing it may be their only income.
- Priorities are determined by weather conditions and the time of year,
- social and religious attitudes within the community and, of course, judgment as to which of the two activities is more remunerative at a particular time.
- Harvesting of agarophytes is done through in-shore collection during low tide,
- on the shores of neighbouring islands and by diving from boats when the seaweed is further out.
- Hand picking of seaweed is normally carried out by women and children equipped with divers' masks and a net bag.

- Metal scrapers have been in use in some areas in recent years and have made it possible to harvest larger quantities of seaweed with less effort,
- but the landed seaweed tends to contain a higher proportion of rock and coral fragments which are scraped up along with the weed.
- In addition, removal of the rootstock prevents regeneration, with the consequent threat to future supplies.
- The collection of alginophytes is generally done by men since it involves picking larger quantities of weed.
- Harvesting *Sargassum* and *Turbinaria* is rather easier than red seaweeds and landings are less likely to contain those unwanted seaweeds which are so difficult to avoid when collecting agarophytes.

Prices for seaweed paid by processor to agent.

Seaweed	*Price paid*

(Rs./tonne, wet wt.)

- *Gelidium/Gelidiella* 5,000— 8,000
- *Gracilaria'* *2,500*— 3,500
- *Sargassum/Turbinaria* 750— 1,000

PRODUCTION OF AGAR IN INDIA

- **Uses and types of agar produced**
- The most important attribute of agar is its great gelling power and the wide range of conditions under which it retains this property.
- Although, as has already been stated, Indian agar has a gel strength lower than that from other sources, it neverthless meets a significant part of the domestic food and pharmaceutical industries' demand.
- Agar is used in the preparation of jellies, dairy products such as yoghurts, confectionery of the jelly/marshmallow type, bakery products, including pie fillings and icings, and canned meats.

- In India, it is widely used in such vegetarian foods and dishes as faluda and blancmange.
- The Muslim community also traditionally consumes large quantities during the Ramadan season.
- Although small amounts of agar may find use as a laxative or excipient,
- the major application of 'pharmaceutical' grade agar is as a culture medium for the growth of micro-organisms such as bacteria and fungi. In this context, pharmaceutical, or **'IF',** grade agar,
- sometimes referred to as 'bacteriological' or 'microbiological' grade, is also used by university and other research establishments and laboratories.
- (The term 'IF grade', used to denote the fact that the agar meets Indian Pharmacopoeia standards, is used hereafter in this report since it is used widely within the Indian agar industry.)
- Its related use as a medium for plant tissue culture is more recent, but one which is gaining an increasing market.
- Among the other minor uses of agar is the preparation of casting moulds, especially those used in dentistry.
- The chemical fraction largely responsible for the gelling properties of agar is agarose, and it finds specialized use in laboratories carrying out biochemical separations.
- However, to date, there has been no Indian production of agarose, the small domestic requirement being met by imports.
- Agar is produced in several different physical forms, the most common being the mat, or strip, form, which is the simplest to produce.
- Where there is a particular requirement on the part of the end-user for high gel strength food-grade agar,
- it is produced in the form of shreds or 'individuals', the latter essentially being strands of larger dimensions than the shreds; both are produced to order, using *Gelidium,* rather than *Gracilaria,* as the raw material.

- Powdered agar, both IF and food grade, is produced from the mat form by milling.

Processing methods employed

- **GENERAL PRINCIPLES**
- The steps required to obtain agar from seaweed is summarized below:
- 1. Cleaning of seaweed
- 2. Chemical pre-treatment
- 3. Extraction of seaweed
- 4. Filtration and gelation of extract
- *5.* Bleaching and dewatering of gel
- In speaking about agarophytes in general terms, it is important to appreciate that what may be optimum conditions for recovery of agar from one source are often not the case from another.
- This is particularly true for chemical pre-treatment and arises because agar is not a homogeneous material.
- Being a mixture of polysaccharides, the proportions of which vary from source to source, the conditions for their extraction also differ.
- Thus, *Gracilaria* requires slightly less severe conditions of processing than *Gelidium* or *Gelidiella*, while *Gracilaria verrucosa*, used by a few producers, needs to be handled differently from *Gracilaria edulis* in certain respects.
- Furthermore, the quality of the final product is highly dependent on the quality of the raw material, which, in turn, is dependent on a number of factors:

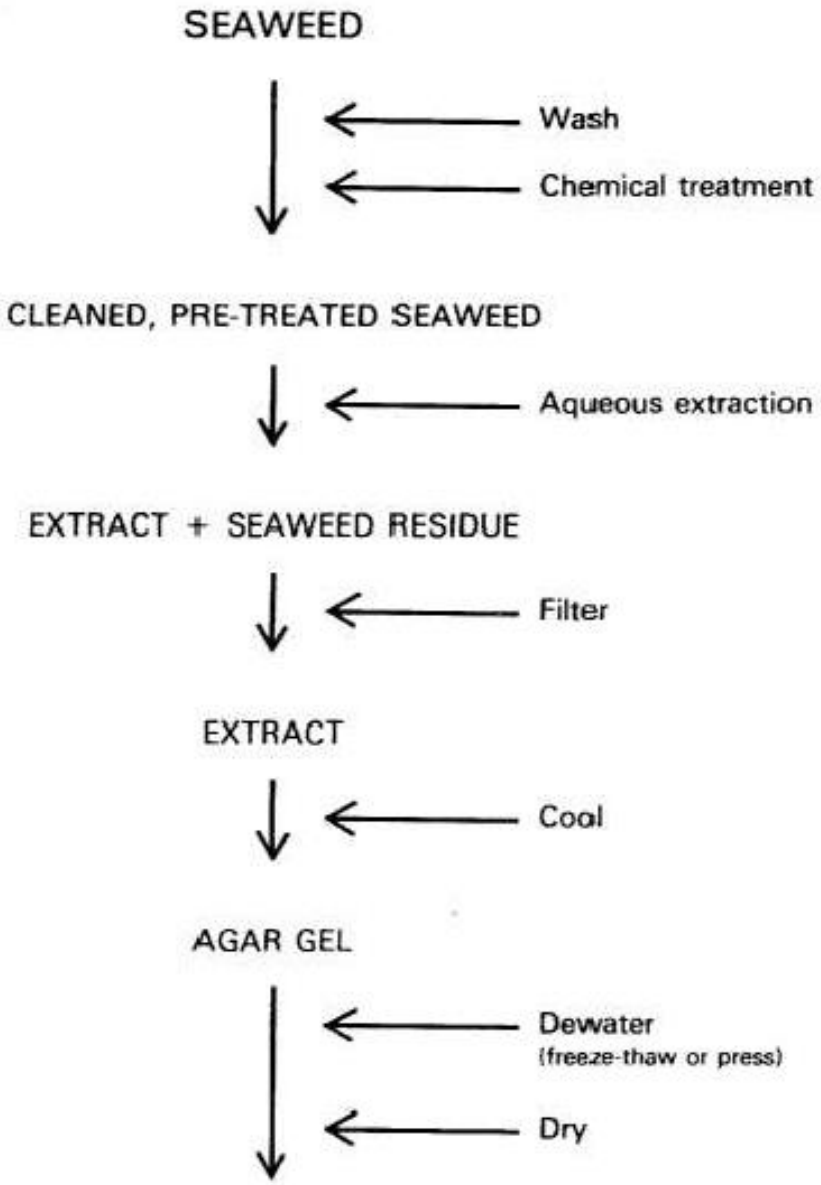
— intrinsic *(e.g.* species of seaweed),

— environmental *(e.g.* temperature and salinity of water during growth of seaweed),

— harvesting *(e.g.* degree of mixing with other weeds), and

— post-harvest *(e.g.* conditions of storage of seaweed prior to processing).

- In India, the same basic method of processing is followed, almost without exception, by all producers: acid pre-treatment of the cleaned seaweed followed by hot water extraction,
- primary dewatering and purification of the agar gel by means of a freeze-thaw cycle, bleaching and, then, sun-drying.



: General processing scheme for production of agar

CLEANING OF SEAWEED

- Agar is insoluble in cold water and the seaweed may safely be washed with water to remove soluble impurities, such as salt, as well as to assist in the separation of foreign matter, such as other weeds, sand, stone and pieces of coral.
- If it has not already been done, the processor, on receipt of the seaweed, would first lay the weed out in the sun to allow some natural bleaching to take place.
- It is then washed as many as four or five times, either briefly or given a more thorough soaking overnight, and dried one or more times between washes.
- Washing is carried out in open cement 'tanks', with or without some form of mechanical agitation, and is accompanied by hand-sorting to remove epiphytes.

CHEMICAL PRE-TREATMENT

- Chemical treatment of agarophytes prior to extraction often produces a better agar in terms of quality or yield than one produced without such treatment.
- Alkali pre-treatment has been found to be the most useful, particularly for *Gracilaria spp.*, a product of higher gel strength being obtained.
- Conditions must be optimized to avoid dissolution and degradation of the agar and so minimize accompanying reductions in yield.
- The chemical rationale of alkali treatment is that increased gel strength results from production of 3, 6- anhydrogalactose units induced by alkaline hydrolysis of galactose-6-sulphate groups within the agar molecule.
- Having said this, it appears that Indian *Gracilaria* does not respond well to alkali treatment.
- Acid pre-treatment, however, has also been recommended or described in the literature and this type of treatment is almost universally employed in India.
- The purpose of the acid is to soften the weed and prepare it for extraction.
- Treatment is accomplished by immersing the weed for 10-15 minutes — and never more than 30 minutes — in cement tanks containing the dilute acid, usually hydrochloric.
- With longer immersion thereis the risk of extracting some of the agar; the remaining agar is also said not to gel well.

- Where *Gracilaria* and *Gelidium/Gelidiella* are used as a mixed raw material, the latter is given a longer acid treatment than the former
- The seaweed is then washed two or three times to free it of acid and transferred to the extraction vessel.

EXTRACTION

- Agar is extracted from seaweed using hot water. Although adjustment of the pH is sometimes beneficial, and the use of certain additives, such as phosphates, is claimed to improve yields or colour, the Indian practice is simply to boil the seaweed in water at normal pressure without the addition of chemicals.
- In this way, the need for sophisticated equipment is eliminated and production costs are kept to a minimum.
- The seaweed is usually added to boiling water in the extraction vessel, which is heated either by live steam introduced through pipes at the bottom of the vessel or, in the case of some of the smaller producers, by direct heat from a wood fire.
- The vessel may be of aluminium, stainless steel or wood, and is of a size to accomodate anything from 20-30 kg of seaweed (+ water) up to 300 kg and more for the largest producers.
- The ratio of water:weed varies from around *5:1* to 10:1 and reflects, to some degree, the raw material used.
- With only one or two exceptions, the seaweed extracted in India is in whole form, since the possible benefits of greater extraction efficiency brought about by reduction of the weed to a finely divided state are more than offset by the difficulties in filtration and separation of the liquor from the mucilaginous residue.
- Even where this is not the case, the weed remains unground; rather, it is pulverized by wooden beaters during the initial washing stages and reduced to pieces a few centimetres in length.
- After addition of the seaweed, boiling is continued for between and 3 hours, the exact time being a matter of judgement on the part of the operator in charge of the extraction.

- He usually removes samples of extract and weed at intervals and tests them with his fingers; good gel formation and the right 'feel' when the weed is pressed between the fingers indicate that extraction is complete.
- The length of time needed is determined in large measure by the quality and nature of the raw material. *Gelidium* requires somewhat longer than *Gracilaria* for complete extraction.
- The state of maturity of the seaweed is also important.
- Yields of agar are not high, usually in the range 5-10% (based on 30-50% purity of the raw weed), though they may be slightly higher for *Gelidium* than *Gracilaria.*
- Such yields are an indication, in part, of the generally poor quality of the seaweed available in India.

FILTRATION AND GELATION OF THE EXTRACT

- On completion of the extraction, the hot aqueous extract is allowed to drain through a metal screen at the bottom of the holding vessel.
- The screen retains the bulk of the seaweed residue and allows the liquid extraction to pass through several layers of filter cloth.
- These remove finer, particulate matter.
- The extracted seaweed, sometimes after recovery of a further, small amount of agar by a second extraction, is discarded as waste or given to local people for use as manure.
- The clarified liquid is then led into shallow aluminium trays, either directly, *via* a hose or buckets, or after temporary storage in a heated tank.
- For the production of IP grade agar, where absolute clarity is required, the liquor is further passed through a filter press before discharge into the trays.
- On setting aside to cool, a firm gel of crude agar soon forms in the trays.

BLEACHING AND DEWATERING OF THE GEL

- Removal of water from the gel, though technically simple, is the most demanding part of the process in terms of time and energy requirements. It is possible to remove much of the water by the application of a gradual and

increasing pressure on the gel, but although this principle forms the basis of the cottage-scale process for crude agar production currently being developed by BOBP, it has not been widely adopted on an industrial scale.

- Instead, in India, as in many other producing countries, the long established method of freezing and thawing the gel is universally used. The freeze-thaw cycle is critical not only to the successful dewatering of the gel but to the attainment of a good quality product as well, the cold thaw water, in which the agar is insoluble, removing low molecular weight polysaccharides, salts and pigments.

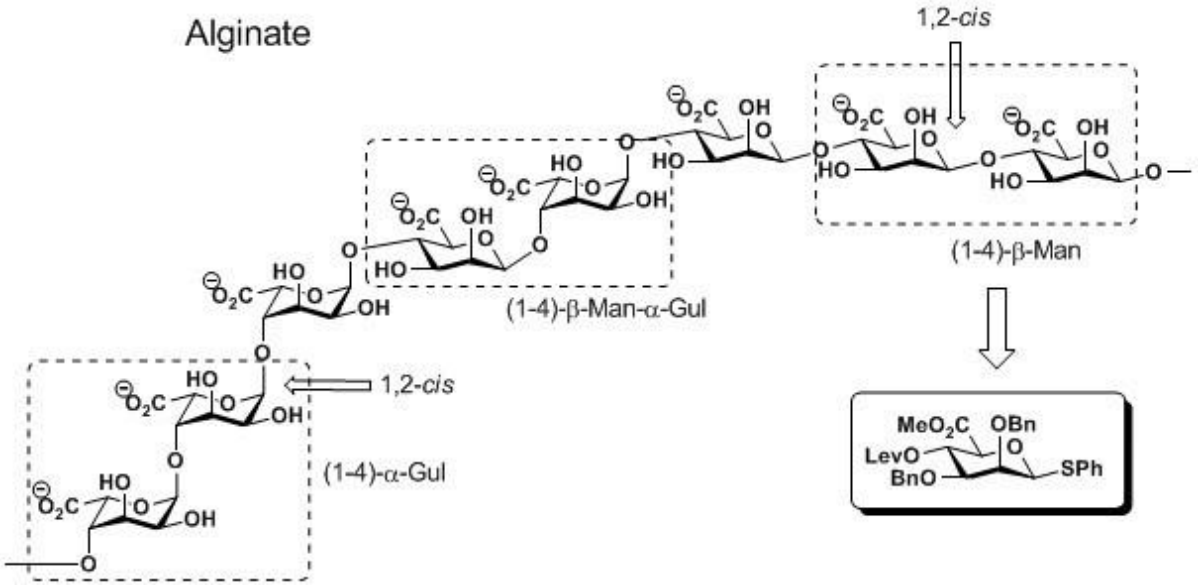
- Within a few hours of forming a gel (no more than 24 hours, otherwise the gel deteriorates) the trays of agar are placed in a freezer, where they are stored for periods of at least 20—24 hours (and sometimes as long as 72 hours) at temperatures ranging from — 10 to — 20°C. The process of freezing should be slow rather than rapid so that a uniformly frozen gel matrix is obtained. To help achieve this, the agar gel is 'combed' with a metal 'comb' before placing it in the freezer; this also makes the subsequent washing and drying more efficient an4 gives the finished product its characteristic mat-like appearance. If the agar is to be produced as 'individuals' or shreds, the cutting or shredding of the gel is done prior to freezing

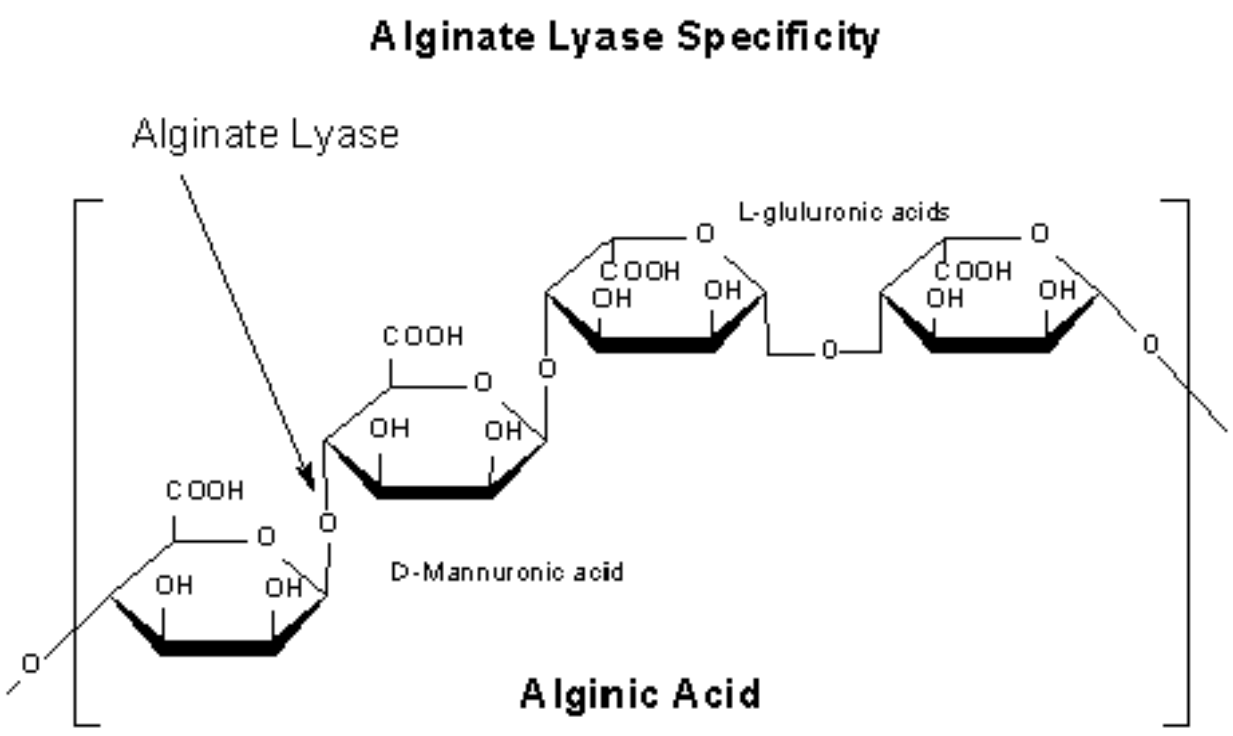
- After removing the trays from the freezer, the agar is allowed to thaw, then washed with water and bleached, usually by immersion in a shallow cement tank containing hypochlorite solution. Treatment is brief, generally no more than 10— 15 minutes. The agar is washed again several times, before finally being laid put on mesh screens to dry in the sun. Occasionally, to obtain a product of low and more consistent moisture content, the sun-dried agar is further dried in a hot-air drier.

- For IP grade agar, deionized water is used for washing and particular care is taken during handling and drying to avoid contamination by specks of dirt and other foreign matter. Agar discolours somewhat on storage, so, if there is no likelihood of immediate sale, bleaching is sometimes omitted at the usual stage and carried out on the dried product only when required . If the agar is to be sold in powdered form, the dried mats of agar are ground and sieved before bagging.

PRODUCTION OF ALGINATES IN INDIA

- Alginic acid is present in seaweed mainly as calcium salt, with lesser amounts as magnesium, sodium and potassium salts.
- The most important commercial derivative *of* alginic acid is the sodium salt, the form in which it is extracted from the seaweed.
- Other derivatives produced include the potassium, ammonium and calcium salts, propylene glycol alginate and alginic acid itself.





The ability of alginates to increase the viscosity of solutions to which they are added in low concentration enables their use in a wide range of applications in the industrial, food and pharmaceutical industries

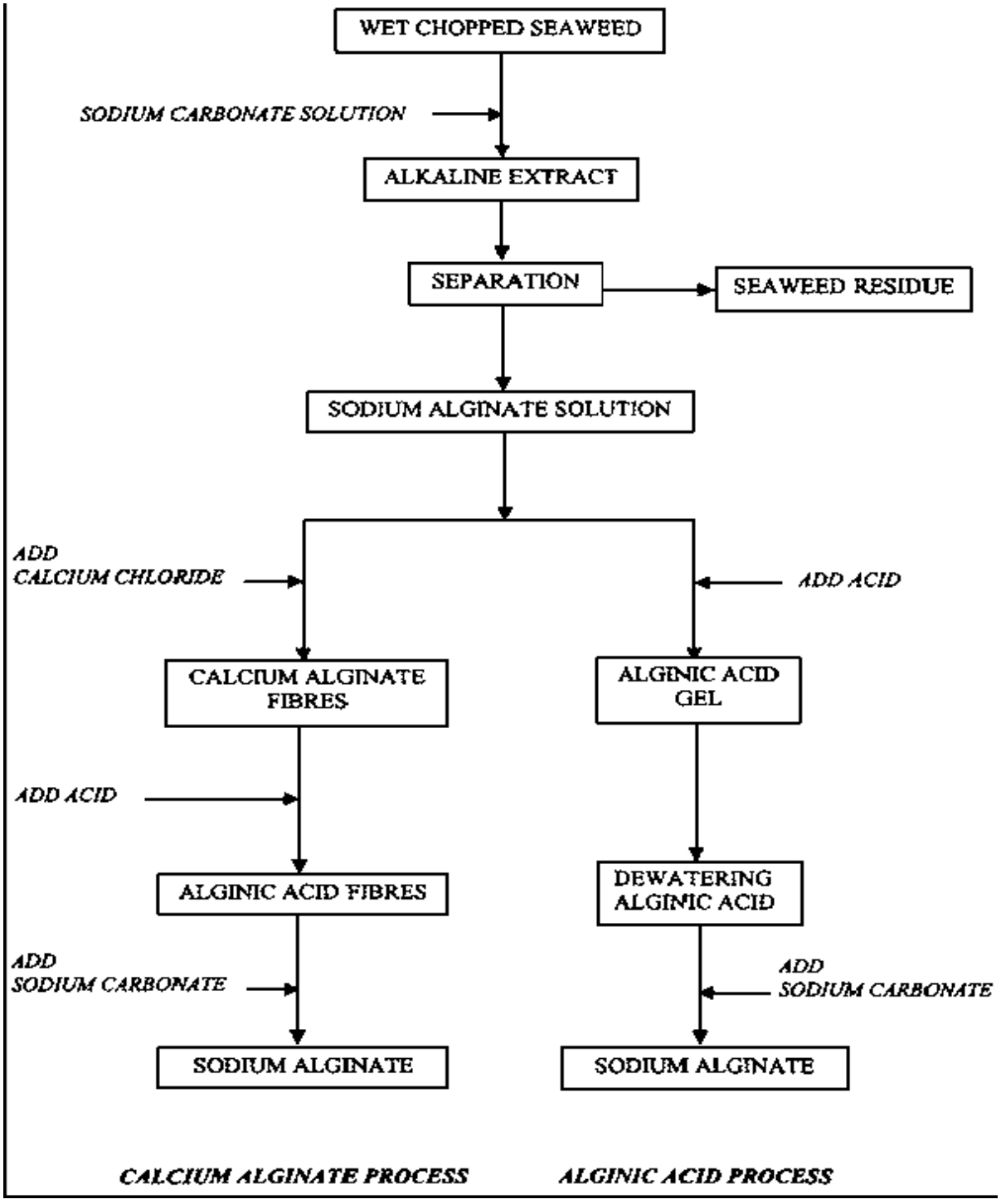
- Of primary importance in India is their use as a thickening printing, agent in textile where they are added to the paste containing the dye.
- Other industrial applications include the manufacture of rubber compounds and paper
- In the food industry, alginates are used in the manufacture of a multitude of and other dairy, bakery, meat products, which take advantage of their thickening, gelling and stabilizing properties.

Processing methods employed

1. Cleaning of seaweed,
2. Chemical pre-treatment,
3. Extraction of seaweed,
4. Separation of extract, then,

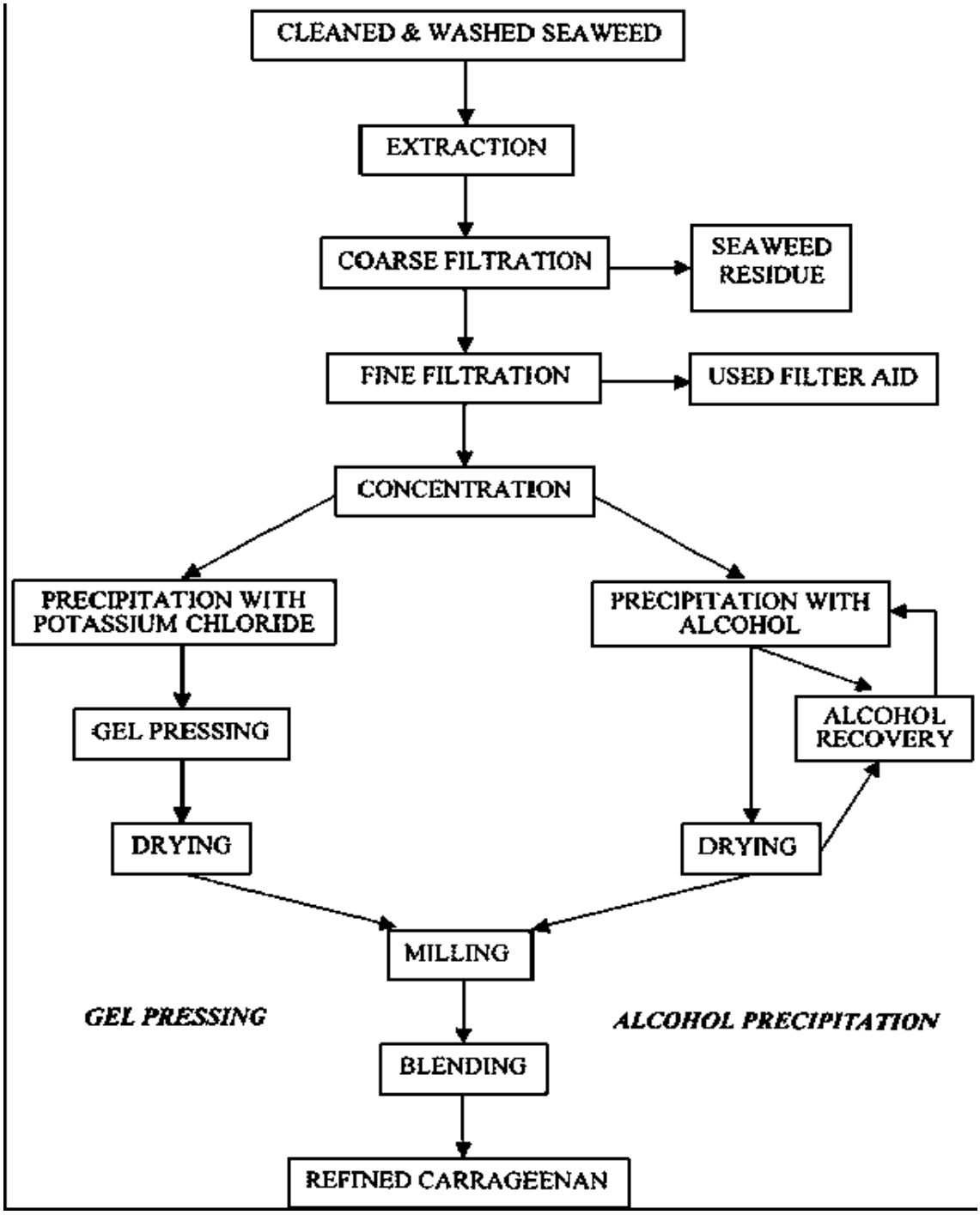
5. Acidification/precipitation of alginic acid, and

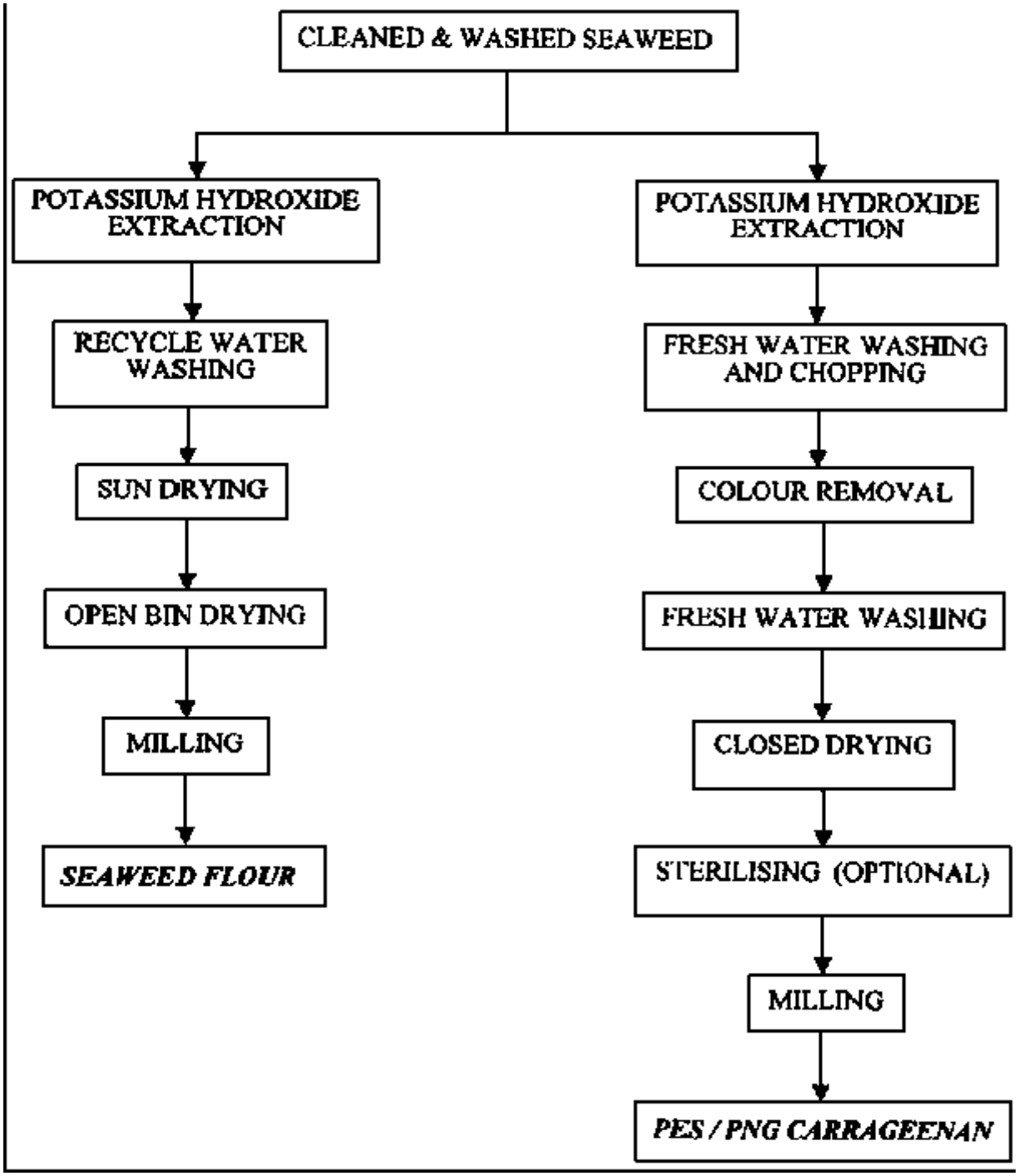
6. Production of sodium alginate



CARRAGEENAN

Chondrus crispus	mixture of kappa and lambda.
Kappaphycus alvarezii	mainly kappa.
Eucheuma denticulatum	mainly iota.
Gigartina skottsbergii	mainly kappa, some lambda.
Sarcothalia crispata	mixture of kappa and lambda.


CLEANED & WASHED SEAWEED
EXTRACTION
COARSE FILTRATION
SEAWEED RESIDUE
FINE FILTRATION
USED FILTER AID
CONCENTRATION
PRECIPITATION WITH POTASSIUM CHLORIDE
PRECIPITATION WITH ALCOHOL
GEL PRESSING
ALCOHOL RECOVERY
DRYING
DRYING
MILLING
GEL PRESSING
ALCOHOL PRECIPITATION
BLENDING
REFINED CARRAGEENAN



- *Kappaphycus alvarezii* is used in this process because it contains mainly kappa carrageenan and this is the carrageenan that forms a gel with potassium salts.
- Iota-containing seaweeds can also be processed by his method, although the markets for iota carrageenan are significantly less than those for kappa.

- Lambda carrageenans do not form gels with potassium and would therefore dissolve and be lost during the alkali treatment.

Carrageenan uses Dairy

products

- The main applications for carrageenan are in the food industry, especially in dairy products.
- Frequently, only very small additions are necessary, 0.01-0.05 percent.
- For example, kappa carrageenan (at 0.01-0.04 percent) added to cottage cheese will prevent separation of whey, and
- a similar amount added to ice cream also prevents whey separation that may be caused by other gums that were added to the ice cream to control texture and ice crystal growth.
- The cocoa in chocolate milk can be kept in suspension by addition of similar amounts of kappa; it builds a weak thixotropic gel that is stable as long as it is not shaken strongly.
- Dry instant chocolate mixes, to be mixed with water or milk, can have improved stability and mouth feel using lambda or a mixture of carrageenans.
- Lambda or a mixture can also improve liquid coffee whiteners by preventing the separation of fat; these applications require 0.2-0.3 percent additions, but much smaller quantities will prevent fat separation in evaporated milks.
- Those small containers of sterilized milk found in the refrigerators of some hotels may have kappa added to prevent fat and protein separation.
- Lambda or kappa may be added to natural cream to help maintain the lightness (incorporated air) if it is whipped.

Water-based foods

- With the appearance of bovine spongiform encephalopathy (BSE, or mad cow disease) and foot-and-mouth disease, efforts have been made to find suitable substitutes for gelatin.

- Gelatin jellies have long been favoured because they melt at body temperature, giving a smooth mouth feel and easy release of flavours. However, if they are stored for a day or two, they toughen and are less pleasant to eat.
- Gels made from iota carrageenan have the disadvantage of a high melting temperature, so they are not as smooth to eat as gelatin gels. They do not melt on hot days and do not require refrigeration to make them set, so these are advantages in hot or tropical climates, and a further advantage is that they do not toughen on storage.
- Carrageenan producers find that by combining various carrageenans with locust bean gum, konjac flour and starch, they can provide a variety of melting and non-melting gels and gel textures to meet the requirements of most of their clients.
- Conventional fruit jellies are based on pectin and a high sugar content to help set the jelly. In a low- or non-calorie jelly the pectin must be replaced, and mixtures of kappa and iota have proved to be suitable.
- Fruit drink mixes to be reconstituted in cold water contain sugar (or aspartame), acid and flavour. Addition of lambda carrageenan gives body and a pleasant mouth feel.
- Sorbet is a creamy alternative to ice cream with no fat; use of a mixed kappa and iota together with locust bean gum or pectin provides a smooth texture to the sorbet.
- Low-oil or no-oil salad dressings use iota or kappa to help suspend herbs, etc., and to provide the mouth feel that is expected from a normal salad dressing.
- The low oil content of reduced-oil mayonnaise normally gives a thin product, rather like a hand lotion; additives are needed to thicken it and to stabilize the oil-in-water emulsion.
- A combination of carrageenan and xanthan gum is effective.
- Xanthan gum is made by a bacterial fermentation process; its development was pioneered in the early 1960s by the Kelco Company, then the largest producer of alginate; it is now an accepted and widely used food additive.
- The interaction of carrageenan and protein can be used in the clarification of beer, with the complex formed precipitating from the wort.

Meat products

- In preparing hams, addition of carrageenan to the brine solution used in pumping improves the product because the carrageenan binds free water and interacts with the protein so that the soluble protein is retained.
- For successful penetration, the brine solution must have a low viscosity, but dissolved carrageenan would increase the viscosity.
- The carrageenan is therefore dispersed in the water after the brine salts are added; the carrageenan does not dissolve because of the high salt concentration, but as the ham cooks it does dissolve and is then effective.
- There is a growing consumer demand for pre-cooked poultry products such as chicken and turkey pieces.
- Poultry processors were concerned about the loss of water during cooking (this lowered their yield per unit weight of product) and the loss in texture and eating quality that resulted.
- By injecting a brine containing salt, phosphate and carrageenan into the muscle of the meat, these problems are overcome.
- As the meat cooks, the carrageenan binds water within the poultry muscle and improves texture and tenderness.
- The processors are pleased because they now have a higher yield; in fact they find that he can even add some extra water to the poultry and it will be retained.
- The consumer receives a better product.
- Hydrocolloids are being tried as fat replacements in low-fat products, with varying degrees of success.
- When fat or salt are reduced, meat and poultry can suffer loss of tenderness, juiciness and flavour.
- Low-fat products formulated with phosphates and carrageenan can have the juiciness and tenderness restored.
- Kappa carrageenan has been used with some success in replacing half the normal fat in frankfurters.

- Reduction of fat in ground meat products like hamburgers results in a different mouth feel and dry taste, which consumers do not always accept.
- Iota can be mixed with fresh ground beef and when cooked it provides fat-like characteristics and moisture retention that make the product more acceptable. This was the basis for McDonald's "MacLean" hamburger.

Air freshener gels

When you need to improve the odours in your room, air freshener gels are one of the products available at supermarkets. They are made from kappa carrageenan, a potassium salt, water and perfume. When mixed, the perfumed gel forms and it is moulded to a shape to fit the holder.

Toothpaste

The essential ingredients in toothpaste are chalk or a similar mild abrasive, detergent, flavour, water and a thickening agent that will provide enough body to the paste to ensure that the abrasive is kept in suspension and that there is no separation of water.

Iota carrageenan, at about 1 percent, is one of the most useful thickening agents.

Immobilized biocatalysts

- Carrageenan gels are another medium for immobilizing enzymes or whole cells.
- Kappa carrageenan gives the strongest gels and beads made from this show sufficient mechanical strength for packing in columns, and yet they are permeable to most substances.

Carrageenan markets

Application	**Tonnes**	**%**
Dairy	11 000	33
Meat and poultry	5 000	15
Water gels	5 000	15
Food grade	8 000	25
Toothpaste	2 000	6
Other	2 000	6
Total	33 000	100

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HEPARIN

Thrombosis, which is a condition created by a formation of a clot inside a blood vessel, has been reported as the leading cause of death. It is also reported that the death rate due to thrombosis is almost twice the rate of death due to cancer, which is the second leading cause of death. Heparin is a commercially available drug that is being used for thrombosis. Despite its universal use in clinical practice, heparin induces some adverse reactions including dependence of antithrombin for anticoagulant action, the need to monitor its effect, lack of general availability of antifactor Xa assays, and its potential to develop heparin-induced thrombocytopenia (HIT syndrome). These adverse effects led many researchers to experiment with novel anticoagulant compounds, especially from natural resources.

With the well-reported history of marine algae containing biologically active substances affecting blood clotting and fbrinolysis, Scientists investigated the potential anticoagulation activity of three types of marine algae: *Pachymeniopsis elliptica, Sargassum horneri,* and *Ulva pertusa* representing the three algae divisions of rhodophyta, phyophyta, and chlorophyta, respectively. An ideal anticoagulant should be efective, safe, non-toxic, independent of vitamin K action, bioavailable when orally administered, and low in cost of production. Methods of extracting bioactive compounds have been subjected to much discussion in research due to its non-viability in an industrial environment. Various methods have been tried out in obtaining bioactive compounds from plant and animal tissues. Screening of anticoagulant compounds from seaweed has been studied using organic solvents, hot water extraction, alkaline and acid digestion and enzymatic extraction methods. These methods have several limitations such as some toxicity effects, high cost and complex procedures. It has been reported that enzymatic extracts from seaweeds possess water-solubility and safety, as this method does not adapt any organic solvent or other toxic chemicals. Moreover, it gains high yield when compared with organic extracts. Although, the enzymatic digestion gives high bioactive compound yield, its substrate specificity, pH adjustments, and residual effects increase the production cost. Few reports on production of methane, organic acids or volatile compounds during seaweed fermentation are available. However, screening of bioactive compounds from seaweeds by natural fermentation method has not yet been studied and developed. Therefore, during past few

years, some laboratory has been carried out fermentation process to convert seaweed macromolecules to different bioactive compounds.

Recently alternative drugs for heparin are in high demand due to its bad and long-term side effects. Therefore, as an alternative source, seaweed polysaccharides gain much attention in the pharmaceutical industry to develop better and safe drugs with low or less side effects. As a cost effective method of digestion, we performed natural fermentation using non-specific microorganisms. In this study, fermentation has been tested as a method of obtaining the potential biologically active compounds, especially with respect to the anticoagulation.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.Tech. BIOTECHNOLOGY

UNIT – V - MARINE BIOTECHNOLOGY – SBT1304

UNIT V: Environmental Impacts of Aquatic Biotechnology (12 hrs)

Human impacts on marine microbial diversity - Usage of marine microbes to ameliorate environmental deterioration. Control of oil spills and bioremediation. Effects of bio-fouling and bio-deterioration on marine structures. Protection methods against corrosion and fouling. Red tides: Causative factors and effects on the organisms of marine environment.

HUMAN IMPACTS ON MARINE MICROBIAL DIVERSITY

Over six billion people now populate the world, and the impacts of our activities are felt in every corner of the globe, including the oceans. The biodiversity of marine microbes is likely to be affected by human activi-ties on both global and local scales.

On the global scale, all organisms in the oceans, including microbes, will respond to the chief fea-ture of global climate change: temperature changes.Elevated temperatures have been found to bring about increases in the numbers of certain waterborne pathogens.

Higher temperatures can also trigger the activity of cer-tain microbial genes, including virulence genes that can lead environmental pathogens to cause disease in humans, corals, and other macroorganisms

Temperature changes in the oceans may also disturb the delicate balance between the numbers of bacteria and phages. The checks and balances system between viruses and their bacterial hosts has recently been recognized as a determinant of bacterial abundance and diversity and of the efficiency of carbon cycling in a given microbial community. It is possible that climate change may not have drastic out-ward effects on the balance between bacteria and phage, however, given the flexibility of the system and the adapt-ability of microorganisms.

On a local level, the ozone hole over the Antarctic, a conse-quence of human releases of ozone-depleting chemicals, is probably having effects on microbial populations there. The gap in the ozone layer allows high levels of ultraviolet rays to reach the surface of the ocean, and is likely to increase micro-bial mutation rates and change microbial community compo-sition in Antarctic waters.

Local impacts also include the release of exotic ballast water. The practice of ballast water dumping is known to release both invasive macroorganisms and potentially harmful microorganisms in coastal waters.

Nutrient loading, whether from fish farming activities or from runoff, has had massive impacts on coastal environments. Fish farms have proven responsible for eutrophication (addi-tions of high concentrations of nutrients) of surrounding waters, resulting in large changes in the local microbial com-munities. Fish farming activities and runoff together have resulted in an increase in the frequency of coastal phyto-plankton blooms, including harmful algal blooms that cost fishermen and governments millions of dollars every year.

Large-scale harvesting of the marine macrofauna by humans has changed the food webs of the oceans and is likely to be affecting marine equilibria and microbial diversity, driving the numbers of some species up and others down. In Peru in the 1970s, for example, over-fishing led to large phytoplankton blooms and a succession of bacteria that created large zones of depleted oxygen, called “dead zones,” in coastal waters. In this case, human activities led directly to a fundamental and devastating change in the microbial ecosystem. The exact nature of the effects of food web changes resulting from over-fishing is little understood and requires further study.

CRITICAL MICROBIALLY-MEDIATED EQUILIBRIA THAT IMPACT ENVIRONMENTAL AND HUMAN HEALTH

While it is true that marine microbial systems drive the biogeo-chemical cycles that make life possible on this planet, marine microbes also have more direct, immediate effects on human health and the well-being of the ocean ecosystem. Many of these direct effects are the result of fragile microbial equilibria, balancing points between opposing trends that could lead to serious repercussions for humans and our environment.

The equilibrium between bacteria and viruses in the oceans is an example of the kind of critical balancing act microbes per-form every day. Changes in water temperature and ultraviolet radiation (UV), two factors known to be impacted by human activities, are known to disturb the relative numbers of bac-teria and viruses in the oceans, with possibly disastrous results for human health. The virulence of a virus that preys on the bacterium responsible for cholera (*Vibrio cholerae*), for example, is affected by subtle changes in temperature. Hence, coastal temperature is a key determinant of the burden of cholera in coastal waters—an important matter to humans who live near those areas. Studies indicate UV can convert viruses between active and dormant forms, so atmospheric changes that increase the amount of light in these wave-lengths that reaches the oceans can upset the balance between viruses and their hosts, possibly leading to uncon-trolled epidemics in fish, invertebrates, or humans.

Microbial communities in the oceans also maintain the balances that can keep harmful algal blooms in check. Algal blooms can poison humans and wildlife that consume shellfish tainted by the algae. It is now known that nutrient pollution can disturb coastal marine microbes, trig-gering these blooms.
Symbiotic equilibria between marine microbes and their hosts could also be upset by human activities. Increases in water temperature, for example, compel corals to drive out their bacterial symbionts. Marine microbes mediate the nutrient ratios in seawater, and releases of nutrients from runoff, wastewater treatment facilities, or other sources that upset those ratios can seriously unbalance ecosystem health.

Marine systems are highly connected with one another— more so than terrestrial systems. As a result, altering micro-bially-mediated equilibria in one part of the ocean will often have impacts on adjacent areas and far-flung regions. The coupling between the sediment (called the benthic zone) and water (called the pelagic zone) in coastal areas is particularly tight and changing one of those components will inevitably affect the other. Similarly, the deep ocean collects material from the upper ocean—the two are somewhat separate but inextricably linked.

MODELS FOR STUDYING THE MICROBIALLY-MEDIATED EQUILIBRIA

Model systems are needed for studying the microbially-medi-ated equilibria that relate to human and environmental health. Some candidate models include:

- **Cyanobacteria and cyanophage** would serve as a good model for investigating the balances between hosts and viruses. The **corals** that have been driven north by warm waters that stimulate *Vibrio* toxicity to the coral's symbiotic zoozanthellae would be an excellent system for studying the impacts of human activities on symbioses.

- The **Chesapeake Bay** could be a good model system of a highly-impacted coastal marine ecosystem. Nutrient inputs to the bay have been curtailed, but these restric-tions have not yet resulted in a marked improvement on measures of ecological health.

- **Lightly stratified marine systems**, like the Red Sea, and **highly stratified pristine coastal systems** could serve as good models for comparison against one another to learn about the conditions that can lead to anoxia.

- **Oligotrophic systems** (nutrient-poor) and **eutrophic coastal systems** (nutrient-contaminated) could make good comparative systems for understanding the sus-ceptibility and resilience of equilibria to nutrient loading.

A model of harmful algal blooms is critically needed. The mechanisms and triggering factors behind these phenomena are not known, and a suitable system for study has yet to be found.

Model ballast water systems are also needed. A great deal of water and many billions of microbes are being moved around the world as ballast, but the effects of these activities are little understood.

REVERSIBILITY OF CHANGES IN MICROBIALLY-MEDIATED EQUILIBRIA

Clearly, the critical equilibria that marine microbes maintain can be easily perturbed by human actions, and many of these conditions, like the balances that keep harmful algal blooms in check, are already in a dis-turbed state. However, some of these impacts may be reversible. Nutrient pollution, for example, which affects the equilibria that control algal blooms and nutrient con-centrations in coastal waters, could be controlled by preventing sewage dumping into the ocean and by restoring the wet-lands and salt marshes that filter nutrients in runoff before it reaches the oceanPreventing the emission of ozone-depleting chlorofluorocarbons would allow the hole in the ozone layer over the Antarctic to recover within decades. A more intact ozone layer would prevent a great deal of harmful UV from reaching the oceans and would relieve bacterial-viral equilibria of the adverse effects of UV.

In an effort to limit pathogen releases, water used in fish farming operations could be treated and recycled within the system. Limiting releases to the oceans would lower the numbers of farm-associated pathogens to which wild populations are exposed and would restore local balances between pathogens and hosts. Recycling would also eliminate (or at least curtail) the release of nutrients to local waters. Nitrogen could be removed from farm water using an anaero-bic ammonia process carried out by marine bacteria. Aquaculture without biological and chemical pollution is a worthy goal for the industry.

Humans have impacted marine equilibria in many ways, and the time required for these systems to recover is not known. Education on these topics is necessary to convey the severity of these impacts to the global community so that aggressive steps can be taken to reverse them

USING MARINE MICROBES TO AMELIORATE ENVIRONMENTAL DETERIORATION

The metabolic diversity of marine microorganisms not only makes them useful in biotechnology applications, it makes them versatile tools for addressing environmental problems. One prime environmental application for these organisms is bioremediation—the treatment of chemical contamination using microorganisms. The cleanup of hydrocarbons, specif-ically petroleum products, is an especially pressing matter in the oceans, where accidents aboard oil tankers can release thousands of gallons of oil in a single incident. In some cases, fertilizing the indigenous microbial communities on the affected beaches with nitrogen and phosphorus can speed the

degradation of the spilled oil, but bioremediation options in open water are limited because of difficulties in delivering sufficient nutrients to sustain biodegradation. It is now known that the actions of an entire microbial community are necessary to break down complex organic matter, including petroleum.

A super-organism that could carry out the entire process was sought, but was never iso-lated or designed.

A cluster of genes that carry out the degradation of chlori-nated biphenyls, called the BPH cluster, have been isolated and are now being applied in treating dredged contaminants from the Hudson River estuary.

*

Today, most research in bioremediation in the marine envi-ronment is focused on the organisms already present in the affected areas. For a number of reasons, few efforts are underway to develop engineered microorganisms to address problems of chemical contamination. There are particular problems with respect to releasing microbes for bioremedia-tion into aquatic environments; confining the organisms to the site of concern, for example, would be difficult.

Finally, there is a possibility that marine viruses can be used to control harmful algal blooms in a sort of "viral therapy." Viruses may be isolated from the algal species responsible for blooms, then engineered or otherwise enhanced in the labo-ratory, and released in a bloom. This approach holds great potential for controlling diseases in aquaculture settings.

CONTROL OF OIL SPILS AND BIOREMEDIATION

The Fate of Oil in the Marine Environment

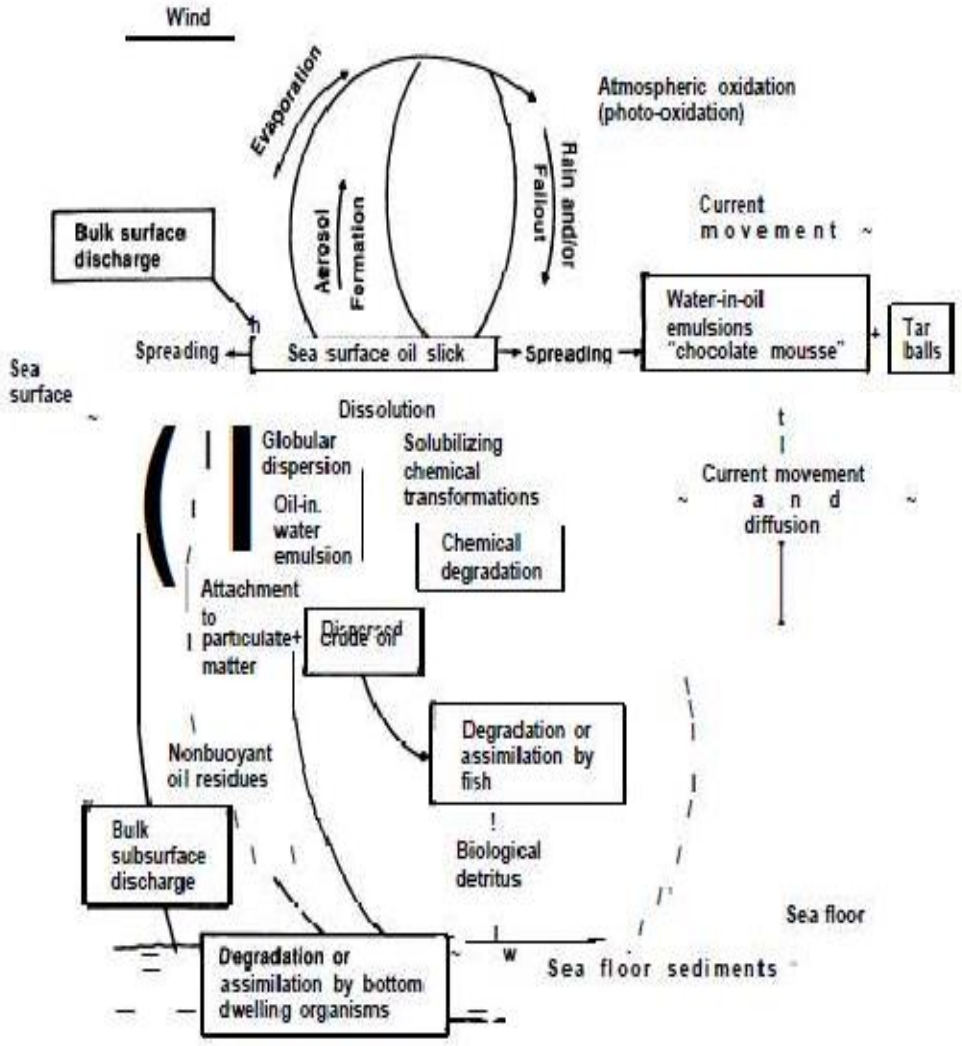
Crude oil and petroleum distillate products introduced to the marine environment are immediately subject to a variety of physical and chemical, as well as biological, changes. Abiological weathering processes include evaporation, dissolution, dispersion, photochemical oxidation, water-in-oil emulsification, adsorption onto suspended particulate material, sinking, and sedimentation. (Figure: 1)

Biological processes include ingestion by organisms as well as microbial degradation. These processes occur simultaneously and cause important changes in the chemical composition and physical properties of the original pollutant, which in turn may affect the rate or effectiveness of

biodegradation. The most important weathering process during the first 48 hours of a spill is usually evaporation, the process by which low- to medium-weight crude oil components with low boiling points volatilize into the atmosphere. Evaporation can be responsible for the loss of one- to two-thirds of an oil spill's mass during this period, with the loss rate decreasing rapidly over time. Roughly one-third of the oil spilled from the Amoco Cadiz, for example, evaporated within the frost 3 days. Evaporative loss is controlled by the composition of the oil, its surface area and physical properties, wind velocity, air and sea temperatures, sea state, and the intensity of solar radiation. The material left behind is richer in metals (mainly nickel and vanadium), waxes, and asphaltenes than the original oil. With evaporation, the specific gravity and viscosity of the original oil also increase. For instance, after several days, spilled crude oil may begin to resemble Bunker C (heavy) oil in composition.

None of the other abiological weathering processes accounts for as significant a proportion of the losses from a spill. For example, the dissolving, or dissolution, of oil in the water column is a much less important process than evaporation from the perspective of mass lost from a spill; dissolution of even a few percent of a spill's mass is unlikely. Dissolution is important, however, because some watersoluble fractions of crude oil (e.g., the light aromatic compounds) are acutely toxic to various marine organisms (including microorganisms that may be able to degrade other fractions of oil), and their impact on the marine environment is greater than mass balance considerations might imply.

Figure I—Schematic of Physical, Chemical, and Biological Processes



Dispersion, the breakup of oil and its transport small particles from the surface to the water column, is an extremely important process in the disappearance of a surface slick. Dispersion is controlled largely by sea surface turbulence: the more turbulence, the more dispersion. Chemical dispersants have been formulated to enhance this process. Such dispersants are intended as a first-line defense against oil spills that threaten beaches and sensitive habitats such as salt marshes and mangrove swamps. Although used widely in other countries, dispersants as have had trouble being accepted in the United States.

The National Research Council has generally approved their use, but effectiveness and, to a lesser degree, toxicity remain concerns. Dispersed oil particles are more susceptible to biological attack than undispersed ones because they have a greater exposed surface area. Hence, dispersants may enhance the rate of natural biodegradation.

Water-in-oil emulsions, often termed “mousse,” are formed when seawater, through heavy wave action, becomes entrained with the insoluble components of oil. Such emulsions can form quickly in turbulent conditions and may contain 30 to 80 percent water. Heavier or weathered crudes with high viscosities form the most stable mousses. Mousse will eventually disperse in the water column and/or be biodegraded, but may first sink or become stranded on beaches. A water-in-oil emulsion is more difficult for microorganisms to degrade than oil alone. Mousse formation, for example, has been suggested as a major limiting factor in petroleum biodegradation of the Ixtoc I and Metula spills, probably because of the low surface area of the mousse and the low flux of oxygen and mineral nutrients to the oil-degrading microorganisms within it . Natural biodegradation is ultimately one of the most important means by which oil is removed from the marine environment, especially the nonvolatile components of crude or refined petroleum.

In general, it is the process whereby microorganisms (especially bacteria, but yeasts, fungi, and some other organisms as well) chemically transform compounds such as petroleum hydrocarbons into simpler products. Although some products can actually be more complex, ideally hydrocarbons would be converted to carbon dioxide (i.e., mineralized), nontoxic water-soluble products, and new microbial biomass. The mere disappearance of oil (e.g., through emulsification by living cells) technically is not biodegradation if the oil has not actually been chemically transformed by microbes.

The ideal may be difficult to reach, particularly in a reasonably short time, given the recalcitrance of some petroleum fractions to biodegradation (discussed below) and the many variables that affect its rate and extent. Man-made bioremediation technologies are intended to improve the effectiveness of natural biodegradation.

Biodegradation and the Chemical Nature of Petroleum Far from being a homogeneous substance, crude oil is a complex mixture of thousands of different chemical compounds. In addition, the composition of each accumulation of oil is unique, varying in different producing regions and even in different unconnected zones of the same formation.

The composition of oil also varies with the amount of refining. Significantly, the many compounds in oil differ markedly in volatility, volubility, and susceptibility to biodegradation. Some compounds are readily degraded; others stubbornly resist degradation; still others are virtually nonbiodegradable. The biodegradation of different petroleum compounds occurs simultaneously but at very different rates. This leads to the sequential disappearance of individual components of petroleum over time and, because different species of microbes preferentially attack different compounds, to successional changes in the degrading microbial community .

Since components of petroleum degrade at different rates, it is difficult and misleading to speak in terms of an overall biodegradation rate. Petroleum hydrocarbons can, in general, be divided into four broad categories: saturates, aromatics, asphaltenes, and resins. Saturated hydrocarbons-those with only single carbon-carbon bonds—usually constitute the largest group. Of these, the normal or straight-chain alkane series is the most abundant and the most quickly degraded. Compounds with chains of up to 44 carbon atoms can be metabolized by microorganisms, but those having 10 to 24 carbon atoms (CIO-C24) are usually the easiest to metabolize. Shorter chains (up to about C12) also evaporate relatively easily. Only a few species can use Cl -C4 alkanes; C5 -C9 alkanes are degradable by some microorganisms but toxic to others. Branched alkanes are usually more resistant to biodegradation than normal alkanes but less resistant than cycloalkanes (naphthenes)-those alkanes having carbon atoms in ringlike central structures. Branched alkanes are increasingly resistant to microbial attack as the number of branches increases. At low concentrations, cycloalkanes may be degraded at moderate rates, but some highly condensed cycloalkanes can persist for long periods after a spill. Light oils contain 10 to 40 percent normal alkanes, but weathered and heavier oils may have only a fraction of a percent. Heavier alkanes constitute 5 to 20 percent of light oils and up to 60 percent of heavier oils. Aromatic hydrocarbons are those characterized by the presence of at least one benzene (or substituted benzene) ring. The low-molecular-weight aromatic hydrocarbons are subject to evaporation and, although toxic to much marine life, are also relatively easily degraded. Light oils typically contain between 2 and 20 percent light aromatic compounds, whereas heavy oils contain 2 percent or less. As molecular weight and complexity increase, aromatics are less readily degraded. Thus, the degradation rate of polyaromatics is slower than that of monoaromatics. Aromatics with five or more rings are not easily attacked and may persist in the environment for long periods. High-molecular-weight aromatics comprise 2 to 10 percent of light oils and up to 35 percent of heavy oils. The asphaltic fraction contains compounds that either are not biodegradable or are degraded very slowly. One of the reasons that tar, which is high in asphaltenes, makes an excellent road paving material is because it is slow to degrade. Tar balls, like mousse, are difficult to degrade because their low surface area restricts the availability of oxygen and other nutrients. Resins include petroleum compounds containing nitrogen, sulfur, and/or oxygen as constituents. If not highly condensed, they may be subject to limited microbial degradation. Asphaltenes and resins are difficult to analyze and, to date, little information is available on the biodegradability of most compounds in these groups. Light oils may contain about 1 to 5 percent of both asphaltenes and resins; heavy or weathered oils may have up to 25 percent asphaltenes and 20 percent resins.

To summarized biodegradation rates are typically highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics, asphaltenes, and resins exhibiting extremely low rates of degradation. As a spill weathers, its composition changes: the light aromatics and alkanes dissolve or evaporate rapidly and are metabolized by microorganisms. The heavier

components that are harder to degrade remain. Weathered Prudhoe Bay oil contains about 10 percent low-molecular-weight alkanes, 45 percent high-molecularweight alkanes, 5 percent light aromatics, 20 percent high-molecular-weight aromatics, 10 percent asphaltenes, and 10 percent resins. Departures from the typical pattern of biodegradation, however, have been noted by some researchers. For example, extensive losses of asphaltenes and resins have been observed in some cases. The microbial degradation of these relatively recalcitrant fractions has been ascribed to co-oxidation.

In this process, a normally refractory hydrocarbon may be partially degraded in the presence of a second readily degraded hydrocarbon. Clearly, degradation rates depend on many factors, and generalizations are difficult to make. One conclusion, however, seems reasonable: no crude oil is subject to complete biodegradation, and claims that all of a light oil or more than 50 percent of a heavy oil can be biodegraded in days or weeks are highly suspect.

Microbial Processes and the Degradation of Petroleum

Despite the difficulty of degrading certain fractions, some hydrocarbons are among the most easily biodegradable naturally occurring compounds. Altogether, more than 70 microbial genera are known to contain organisms that can degrade petroleum components (table 1). Many more as-yet-unidentified strains are likely to occur in nature.

Moreover, these genera are distributed worldwide. All marine and freshwater ecosystems contain some oil-degrading bacteria. No one species of microorganism, however, is capable of degrading all the components of a given oil. Hence, many different species are usually required for significant overall degradation. Both the quantity and the diversity of microbes are greater in chronically polluted areas. In waters that have not been polluted by hydrocarbons, hydrocarbon-degrading bacteria typically make up less than 1 percent of the bacterial population, whereas in most chronically polluted systems (harbors, for example) they constitute 10 percent or more of the total population. Microorganisms have evolved their capability to degrade hydrocarbon compounds over millions of years. These compounds are a rich source of the carbon and energy that microbes require for growth. Before that carbon is available to microorganisms, however, large hydrocarbon molecules must be metabolized or broken down into simpler molecules suitable for use as precursors of cell constituents.

The activity of microorganisms at a spill site is governed by the organisms' ability to produce enzymes to catalyze metabolic reactions. This ability is, in turn, governed by their genetic composition. Enzymes produced by microorganisms in the presence of carbon sources are responsible for attacking the hydrocarbon molecules. Other enzymes are utilized to break down hydrocarbons further. % Lack of an appropriate enzyme either prevents attack or is a barrier to complete hydrocarbon degradation.

The complex series of steps by which biodegradation occurs constitutes a metabolic pathway. Many different enzymes and metabolic pathways, not all of which can be found in any single species, are required to degrade a significant portion of the hydrocarbons contained in petroleum. (Thus, advocates of using specially selected mixtures of microorganisms to bioremediate oil spills or of creating, through recombinant DNA technology, genetically engineered organisms are motivated in part by the desire to combine all the requisite enzymes and pathways.) Knowledge of the numerous metabolic pathways involved in the breakdown of hydrocarbons is far from complete. Additional research characterizing the microbiology and population dynamics of bacterial species capable of degrading oil is critical to
understanding the biodegradation process.

Table I—Major Genera of Oil-Degrading Bacteria and Fungi

Bacteria	*Fungi*
Achromobacter	Allescheria
Acinetobacter	Aspergillus
Actinomyces	Aureobasidium
Aeromonas	Botrytis
Alcaligenes	Candida
Arthrobacter	Cephalosporium
Bacillus	Cladosporium
Beneckea	Cunninghamella
Brevebacterium	Debaromyces
Coryneforms	Fusarium
Erwinia	Gonytrichum
Flavobacterium	Hansenula
Klebsiella	Helminthosporium
Lactobaoillus	Mucor
Leumthrix	Oidiodendrum
Moraxella	Paecylomyces
Nocardia	Phialophora
Peptococcus	Penicillium
Pseudomonas	Rhodosporidium
Sarcina	Rhodotorula
Spherotilus	Saccharomyces
Spirillum	Saccharomycopisis
Streptomyces	Scopulariopsis
Vibrio	Sporobolomyces
Xanthomyces	Torulopsis
	Trichoderma
	Trichosporon

SOURCE: G.D. Floodgate, "The Fate of Petroleum in Marine Ecosystems," *Petroleum Microbiology*, R.M. Atlas (ad.) (New York, NY: Macmillan Publishing Co., 1984), p. 373.

Environmental Influences on Biodegradation

Environmental variables can also greatly influence the rate and extent of biodegradation. Variables such as oxygen and nutrient availability can often be manipulated at spill sites to enhance natural biodegradation (i.e., using bioremediation). Other variables, such as salinity, are not usually controllable. The great extent to which a given environment can influence biodegradation accounts for some of the difficulty in accurately predicting the success of bioremediation efforts. Lack of sufficient knowledge about the effect of various environmental factors on the rate and extent of biodegradation is another source of uncertainty.

Oxygen

Oxygen is one of the most important requirements for microbial degradation of hydrocarbons. However, its availability is rarely a rate-limiting factor in the biodegradation of marine oil spills. Microorganisms employ oxygen-incorporating enzymes to initiate attack on hydrocarbons. Anaerobic degradation of certain hydrocarbons (i.e., degradation in the absence of oxygen) also occurs, but usually at negligible rates. Such degradation follows different chemical paths, and its ecological significance is generally considered minor. For example, studies of sediments impacted by the *Amoco Cadiz* spill found that, at best, anaerobic biodegradation is several orders of magnitude slower than aerobic biodegradation. Oxygen is generally necessary for the initial breakdown of hydrocarbons, and subsequent reactions may also require direct incorporation of oxygen. Requirements can be substantial; 3 to 4 parts of dissolved oxygen are necessary to completely oxidize 1 part of hydrocarbon into carbon dioxide and water. Oxygen is usually not a factor limiting the rate of biodegradation on or near the surface of the ocean, where it is plentiful and where oil can spread out to provide a large, exposed surface area. Oxygen is also generally plentiful on and just below the surface of beaches where wave and tide action constantly assist aeration. When oxygen is less available, however, the rates of biodegradation decrease. Thus, oil that has sunk to the sea floor and been covered by sediment takes much longer to degrade. Oxygen availability there is determined by depth in the sediment, height of the water column, and turbulence (some oxygen may also become available as the burrowing of bottom-dwelling organisms helps aeration) .Low-energy beaches and fine-grained sediments may also be depleted in oxygen; thus, the rate of biodegradation may be limited in these areas. Pools of oil are a problem because oxygen is less available below their surfaces. Thus, it may be preferable to remove large pools of oil on beaches, as was done in Alaska, before attempting bioremediation.

Nutrients

Nutrients such as nitrogen, phosphorus, and iron play a much more critical role than oxygen in limiting the rate of biodegradation in marine waters. Several studies have shown that an inadequate supply of these nutrients may result in a slow rate of biodegradation. Although petroleum is rich in the carbon required by microorganisms, it is deficient in the mineral nutrients necessary to support microbial growth. Marine and other ecosystems are often deficient in these substances because non-oildegrading microorganisms (including phytoplankton) consume them in competition with the oildegrading species. Also, phosphorus precipitates as calcium phosphate at the pH of seawater. Lack of nitrogen and phosphorus is most likely to limit biodegradation, but lack of iron or other trace minerals may sometimes be important. Iron, for instance, is more limited in clear offshore waters than in sediment-rich coastal waters. Scientists have attempted to adjust nutrient levels (e.g., by adding nitrogen- and phosphorus-rich fertilizers) to stimulate biodegradation of petroleum hydrocarbons. This is the experimental bioremediation approach used recently on about 110 miles of beaches in Prince William Sound, Alaska. Researchers have also experimented with alternative methods of ***applying*** nutrients. Given the

necessity of keeping nutrients in contact with oil, the method of application is itself likely to be an important factor in the success of bioremediation.

Temperature

The temperature of most seawater is between –2 and 35^{o}C. Biodegradation has been observed in this entire temperature range, and thus in water temperatures as different as those of Prince William Sound and the Persian Gulf. The rates of biodegradation are fastest at the higher end of this range and usually decrease—sometimes dramatically in very cold climates-with decreasing temperature. One experiment showed that a temperature drop from 25 to 5oC caused a tenfold decrease in response. At low temperature, the rate of hydrocarbon metabolism by microorganisms decreases. Also, lighter fractions of petroleum become less volatile, thereby leaving the petroleum constituents that are toxic to microbes in the water for a longer time and depressing microbial activity. Petroleum also becomes, more viscous at low temperature. Hence, less spreading occurs and less surface area is available for colonization by microorganisms. In temperate regions, seasonal changes in water temperature affect the rate of biodegradation, but the process continues year-round. Other Factors several variables, including pressure, salinity, and pH may also have important effects on biodegradation rates. Increasing pressure has been correlated with decreasing rates of biodegradation; therefore, pressure may be very important in the deep ocean.

Oil reaching great ocean depths degrades very slowly and, although probably of little concern, is likely to persist for a long time. Microorganisms are typically well adapted to cope with the range of salinities common in the world's oceans. Estuaries may present a special case because salinity values, as well as oxygen and nutrient levels, are quite different from those in coastal or ocean areas. However, there is little evidence to suggest that microorganisms are adversely affected by other than hypersaline environments.

Extremes in pH affect a microbe's ability to degrade hydrocarbons. However, like salinity, pH does not fluctuate much in the oceans-it remains between 7.6 and 8. l—and does not appear to have an important effect on biodegradation rates in most marine environments. In salt marshes, however, the pH maybe as low as 5.0, and thus may slow the rateof biodegradation in these habitats.

General Advantages and Disadvantages of bioremediation

Bioremediation technologies have several attributes that, depending on the situation and type of site may support their use in responding to some oil spills (table 2). First, bioremediation usually involves minimal physical disruption of a site. This attribute is especially important on beaches where other available cleanup technologies (e.g., high- and low-pressure spraying, steam cleaning, manual scrubbing, and raking of congealed oil) may cause additional damage to beach-dwelling biota. Application of oleophilic (i.e., oil seeking) fertilizers during the 1989-90 Alaska bioremediation experiments was accomplished largely from shallow draft boats located just off the beach.

Second, bioremediation technologies appear to have no or only minor and short-lived adverse effects when used correctly. Although research on possible negative impacts is continuing, there is so far little evidence to suggest that potential problems would be significant.

Third, bioremediation may be useful in helping remove some of the toxic components of petroleum (e.g., low-molecular-weight aromatic hydrocarbons) from a spill site more quickly than they might otherwise be removed by evaporation alone. Fourth, bioremediation of oil spills is accomplished on-site, and offers a simpler and more thorough solution to polluted areas. In contrast, hot water spraying of an oiled beach, for example, flushes some surface oil back into the water, and this oil must then be recovered by skimmers. The recovered oil-and-water mixture must be separated, and the oil disposed of or recycled. Also, a significant amount of mechanical equipment and logistical capability is required to deal with a large spill.

Because bioremediation equipment and logistics are usually simpler and less labor intensive, costs *may be* lower than for other techniques. At the same time, the total cost of cleanup is the more important concern, and where bioremediation is used as an adjunct or secondary technology, total costs—as well as total benefits--could be greater. The costs of monitoring bioremediation must also be considered. Bioremediation technologies have several general disadvantages. Although bioremediation may work faster-potentially much faster—than natural biodegradation, it cannot produce significant ***short*** *term* results. If beaches are threatened by a large offshore spill, for instance, bioremediation is probably not appropriate as an initial defensive measure.

In this circumstance, it would usually be more appropriate to get the oil out of the water as quickly as possible or, failing this, to disperse or burn it before it drifts onto beaches. bioremediation takes too much time to work as a primary response measure for such a threat. Second, the bioremediation approach must be specifically tailored to each polluted site. Bioremediation technologies are not, and are unlikely soon to become, off-the-shelf technologies that can be used with equal effectiveness in every locale. Although other oil spill response technologies are subject to this same constraint, the advance knowledge needed for bioremediation technologies is greater. Advance knowledge of, for example, the efficiency of the bacteria indigenous to an area in degrading oil, the availability of rate-limiting nutrients, and the susceptibility of the particular spilled crude oil or refined product to microbial attack is required, so prespill planning will be important.

Finally, the public is still unfamiliar with bioremediation technologies. Although public attitudes toward “natural” solutions to environmental problems are generally favorable, the lack of knowledge about microorganisms and their natural role in the environment could affect the

acceptability of their use. Before bioremediation technologies are likely to be widely used, their efficacy and safety will have to be convincingly demonstrated and communicated to the public.

ENVIRONMENTAL ISSUES: EFFECT OF BIOFOULING AND BIODETERIORATION ON MARINE STRUCTURE

MARINE FOULING

Fouling does not destroy materials directly. It is the settlement of marine fouling organisms on all structures made of wood, steel, FRP, aluminium and ferrocement exposed to seawater. Immediately after a substrate is immersed in seawater, fouling settlement starts and the sequence of processes are formation of a primary film or slime film (formed by bacteria, fungi, diatoms and protozoa enmeshed in detritus), fixation of larvae of macroscopic organisms (algae, tubeworms, bryozoans, hydroids, barnacles, mollusks) and finally the growth of the fouling community. In the case of ships because of fouling the roughness of hull and the fuel consumption are increased while speed is reduced. Corrosion process in the marine medium is closely related to the failure of antifouling coatings. Underwater or splash zone of marine structures are subjected to a very harsh environment where corrosion and biofouling combine to cause loss of millions of rupees annually.

Bacteria, fungi, diatoms and algae are the most frequent vegetable organisms attached while hydroids, bryozoa, tunicates, serpulids and barnacles are the animal species generally recorded. Of these the barnacles especially of the genus Balanus are the most aggressive, deteriorating the organic coating, affecting the continuity and favouring corrosion. Variation in temperature, salinity, pH, oxygen content and pollution influence this process.

FOULING CONTROL

A periodical coating of antifouling coatings seem to be the only accepted method for fouling prevention throughout the world. In the AF paint film, biocides must be released during the lifetime of the coating and must cover a wide spectrum of fouling species. Different antifouling coatings were developed over the years according to the type of ship, area of operation of ships, trading speed, vessel activity in days per year, maximum length of stay in port and docking intervals. Fouling normally occurs when a vessel is stationary and does not take place at speeds less than 6 knots.

Cuprous oxide, an inorganic toxicant and tributyl tin oxide (TBTO) is an organic toxicant most commonly used in the antifouling coatings. Paints based on organometallic compounds such as TBTO and tributyl tin fluoride (TBTF) provide 4 to 5 years of fouling free life. However the use of these is restricted due to the problem of environmental pollution.

The awareness of environment in the recent years paved way to the development of alternate coatings and new procedures for fouling control. Considering current antifouling regulation in different countries, the use of coatings requires clearance from the Government. In general, TBT based antifouling paints must not be applied to vessels of < 25 m in length and they must have

biocide release rates less than 4 µg TBT cm -2 day -1. All copper based coatings must have a copper release rate of less than 40 µg cm -2 day -1. Tin free coatings using chemicals such as ammonium quaternary compounds and polymeric silicones bonded to polymeric chain are reported to obtain a low bio-adherence. Development of a system that uses non-stick mechanism to give a glossy surface without biocides is also under study. The incorporation of natural products of plant or animal origin, which have antifouling properties in the antifouling coatings, is a new area of study.

BIODETERIORATION

Biodeterioration or biodegradation of materials can be defined as any undesirable change in the properties of a material caused by the vital activities of biological agencies organisms. Though these are considered synonyms, Starkey (1976) defined biodeterioration as `biological that are destructive or yield undesirable products or both'; and biodegradation as `breakdown of undesirable materials to harmless or tolerable products'.

There are 3 factors to be taken into consideration for prevention of any form of biodeterioration. These are the `material, the environment and the organisms'.

There are different forms of biodeterioration, viz., (i) Biofouling which is in the form of deterioration occurring when the mere presence of an organism or its excrement renders the product unacceptable. (ii) Chemical assimilatory biodeterioration occurring when a material is degraded for its nutritive value. In the field of marine biodeterioration, the important areas are bacterial deterioration, fungal attack, boring problem, biofouling and biocorrosion. In India around 1,80,000 crafts comprising of catamarans, dug out canoes, built up boats and modern mechanised boats are employed for fishing. Of these majority are of wood and a loss of millions of rupees is incurred annually due to biodeterioration of these boats. Wooden objects contain more than 70 % of cellulose which is a good material suited for biological agencies. The bacterial and fungal attack and boring problem are more concerned Biodeterioration with small boats. With the advent of extended voyages, larger vessels made of steel are in use whereby fouling closely associated with biocorrosion became a major problem.

BACTERIAL DETERIORATION

Bacterial deterioration of wooden material is a slow process. Many wood destroying bacteria are able to attack cellulose, but some are capable of attacking lignified cell wall also, particularly when wood is exposed for long time to wet condition. Bacillus Spp., Pscudomonas spp. Vibrio spp., Aerobacter spp. and Aerogenes Spp. were reported from wooden materials. They either inhabit wood or utilize cellulose as food. Bacteria initially colonise the parenchyma cells of wood rays and resin ducts, but later walls of cells are attacked and degraded by cellulase or pectinase activity or a combination of both. The process in deterioration of wooden objects in ground contact is slower, but opening up the pit membranes make gaseous exchange easy. This causes the conditions inside the wood more aerobic suitable for fungal growth and open pathways make fungal hyphae to pass from cell to cell.

When immersed in sea water bacteria initiate the problem of fouling. The initial step in fouling is the bacterial colonization of surfaces followed by attachment of protozoa, fungi and microalgae resulting in `biofilm' formation which is followed by macrofouling.

FUNGAL DETERIORATION

Fungal deterioration Biological deterioration of wood is caused by wood inhabiting fungi both on land and in water. They differ from ordinary green plants in form and method of nutrition. They are unable to produce their own food and are parasitic/saprophytic in nature deriving food from the cell cavities of the host wood. Fungi possess certain enzymes capable of digesting the cellulose. Due to enzymatic action the timber becomes soft and light, spongy, inflammable and emit a mucky and unpleasant odour. Eventually fungal attacked wood gets fully soaked in water and becomes heavy and loses the nail holding properties and strength properties. In dry condition, the wood cracks and these gradually become longer and deeper resulting in the failure of such structures. Fishing crafts have to be periodically repaired or replaced and the cost of this runs to several lakhs of rupees. Hence the problem is of considerable economic importance and proper maintenance is essential.

Based on the development and the type of deterioration they cause on the wood, two types are distinguished

a. wood staining fungi

b. wood destroying fungi

WOOD STAINING FUNGI

Fungi of this group do not destroy the wood but produce certain stains on the surface, which are troublesome because of their objectionable appearance. The wooden objects may take different shades of blue, black, brown and green. Most staining fungi could cause soft rot under prolonged favourable conditions. Protection against such decay can be accomplished by kiln drying or temporarily by surface treatment with a water solution of anti-stain fungicide. Wood destroying fungi Wood destroying fungi are those capable of disintegrating the cell walls and thereby changing the physical and chemical characteristics of wood. Wood undergoes marked change in colour, texture and strength properties and eventually the wood becomes soft and spongy. Well-known wood destroying fungi consist of 2-sub groups, brown rot and the white rot types of Basidiomycetes. Both use cellulose and other carbohydrates as nourishment, but only white rot type is capable of breaking down lignin.

Soft rot fungi belonging to the cellulose-decomposing group are usually the first fungal colonisers of wood. They consume sugars or simple carbohydrates. Zygomycetes, Ascomycetes and Fungi impertectii belong to this group. Usually the attack is superficial and generally occurs in the wooden pieces exposed to high moisture content and on ground contact. In degree of wood deterioration this is intermediate between stain and decay

Factors affecting fungal deterioration

Wood structure: The wood structure viz. the presence of extractives, resins etc. influences the resistance to decay or the natural durability of the timber. Sapwood will be susceptible to decay with a readily available supplementary source of nutrients in the ray parenchyma and the absence of toxic extractives in the cell walls. The heartwood can be highly resistant to decay with the presence of toxic extractives and with the absence of nutrients.

Moisture: Microbiological deterioration can occur only if the wood material has a moisture content exceeding 20 %. Decay fungi and stain fungi can cause severe damage only when the moisture content is above the fibre saturation point (30 %) level but at the same time the development of decay is retarded by excess moisture.

Air: A supply of air is necessary for the growth of wood destroying fungi. An amount of air equivalent to more than 20 % of the volume of the wood must be available before decay can take place. Wood saturated with water is devoid of sufficient air for fungal growth and consequently does not decay.

Temperature: Fungi can grow in wood at a fairly wide range of temperature, about 15 to 30 0 C. The activity decreases at temperature above and below this range and effective growth ceases at about 5 to 10 0 C at the lower limit and 35 to 40 0 C at the higher limit.

Nutrition: Energy for most of the cell building materials for the organisms are supplied by carbohydrate fraction consisting of cellulose, starches and sugars and for some organisms by lignin fraction. The cellulose, hemi cellulose and lignin constitute 95 % of the substance of most woods, sufficiently abundant to meet the requirement of organisms.

pH: All decay fungi produce optimum development at about pH 6, though soft rot fungi grow at pH 8 or 9.

Light: Light is needed for typical sexual reproduction among decay fungi. Effects of fungal decay in woods

i. Alter physical and chemical characteristics of wood
ii. The normal colour is modified and distinctive odours are imparted to wood.
iii. Reduces density
iv. Modifies heat and electrical conductivity of wood.
v. Reduces mechanical strength properties

Boat parts subjected to fungal decay: Salt-water members above waterline are more liable to fungal decay than water exposed surfaces. Stem, transom planks, frame heads, beam-ends and bulwork, stanchion ends and bulkheads are the parts most affected. Poor material (use of sapwoods and unseasoned wood), warm climate, fresh water leaks and dead air spaces are factors responsible for the decay.

Prevention

Taking certain precautions while construction of the boat and during service can prevent the problem. Using decay resistant heartwood seasoned to below 20% moisture level and

avoiding infected wood would resist the problem. All water should be kept out and all the seams should be well caulked. Metal fastenings should be kept tight. Exterior paint will keep out moisture but painting inside the hull planks may be avoided allowing the hull planks to breathe. The moisture that has entered into wood can be got rid of by drying or airing.

Suitable commercial wood preservatives should be used at the time of construction and be repeated periodically while in use. Pentachlorophenol, Copper Chrome Arsenate (CCA) and Creosote are some recommended wood preservatives, which can be applied either by brush application or by pressure treatment. The `dual treatment' incorporating a waterborne preservative such as CCA followed by an oil borne preservative such as creosote gives superior protection to wood against fungal decay.

Marine wood borers

Destruction of wood by marine wood borers is of great economic concern. Wooden structures exposed to marine environment are subjected to attack by a range of wood boring marine organisms designated as `marine borers'. They attack ships, log rafts, harbour piles and many other waterfront structures causing structural damage by boring deep into the wood making them unserviceable within a short span of time. These animals are distributed throughout the salt waters of most of the world but are more prevalent and destructive in the tropics than in the temperate regions.

The marine borers that cause the greatest amount of damage are categorized into two: bivalve molluscs and crustaceans each characteristic in its general appearance and method of attacking wood. The molluscan borers may be separated into two families - the Teredinidae or the wood - boring shipworms, and the pholadidae or rock borers. The important genera of wood boring molluscs are Teredo, Bankia, Nausitoria and Martesia of which the first three are superficially worm like in appearance and are known as `shipworms'. The damage caused by shipworms is internal and can become quite extensive without being apparent. The larvae make very small entrance holes on the surface of the wood, but once within the wood they increase rapidly in size and develop the characteristic worm like bodies. As the animal advances into wood, it secretes a protective calcareous lining for the burrow. As a result of the continued boring, the structural strength may be greatly reduced. Teredo elongata, T. manni, T. furcifera,T. milleri, Bankiella carinata, B. liliobankia and Nausitoria hedlei are the species important to India.

The pholadidae look very much like small clam in appearance and bore into wood, clay, soft rock, shells and even into plastic and poor grades of concrete. Pholadidae is represented by Pholas and Martesia of which Martesia is of importance to India because of its widespread distribution, density of attack and rapid succession of generation. The young attack wood by boring small entrance holes and once within the wood, they continue their boring and excavate the wood sufficiently to accommodate the growth of their imprisoned bodies. M.striata and M.fragilis are common to India.

Crustacean borers are distinct from the molluscan borers in their method of attack, general structure and appearance. They do not become imprisoned in the wood but are able to move about. The young and adult alike attack the wood making narrow galleries, which seldom reach very deep. The damage done by this group is less serious than by shipworms as this is more evident to inspection and the excavation proceeds less rapidly. The animals make extensive network of tunnels in the wood, which are eroded away by wave action, which exposes unattacked surface for fresh attack. The important crustacean borers are of two orders `Amphipoda' and `Isopoda' and are represented by three major genera viz. Limnoria, Sphaeroma and Chelura of which the latter is of minor importance to India. Sphaeroma commonly called as `pill bugs' grow to a size of 13 mm long while Limnoria is much smaller growing to a size of 6 mm only. S. terebrans, S. annandeli, S. walkeri, L. tripunctata, L. bombayensis, L. insulae and L. andamanaensis are active in Indian waters.

Control of marine borers

The degree of resistance to borer attack depends on the species of timber and different localities probably due to the presence of different species of borers. The problem is more pronounced in tropical waters than in temperate waters. Traditionally indigenous preparations such as fish oil, crude oil, cashew nut shell liquid, coconut oil, sand, cement, black tar, fuel oil etc singly or in combination are used for protection. Since most of these preparations lack toxic property, CIFT has recommended treatment of wood with chemical preservatives or use of physical barriers applied to the surface of the timber. Use of Creosote, an oil borne preservative was found to be successful in preventing teredenid attack. Water borne preservatives such as copper-chrome- arsenic (CCA) compounds are very effective against borers especially to crustacean borers. Dual treatment - an initial treatment with a water borne preservative followed by an oil borne preservative (Creosote) treatment - is very effective against both types of borers and is recommended for areas of very severe borer attack.

Physical barriers such as metals (Copper, aluminium etc.), concrete and plastic have been used to achieve protection viz., the hull below the waterline area of boats is sheathed. Instead of copper, which is very costly, aluminium-magnesium alloy has been recommended by CIFT and the sheathing has been standardized. Fibreglass reinforced plastic (FRP) sheathing also is a proven method of protection.

Microbial Corrosion

Seawater is a well-known corrosive environment and any biological activity can enhance its aggressiveness. The involvement of microorganisms in metal corrosion process was suggested as early as in late 1890s. It was reported that the corrosive action of water on lead could be due to ammonia, nitrites and nitrates produced by bacteria. The interaction of a biofilm and the substratum produces a new physical and chemical environment. The conditions at the substratum

will be quite different to that in the bulk phase or in the unfouled surface and the activity of microorganisms within biofilms will result in a range of consequences. .
Mictrobial action may bring about metallic corrosion by one or more of the following mechanisms:
(a) production of corrosion metabolic products
(b) production of differential aeration cells,
(c) disruption of protective films (natural and applied) and breakdown of corrosion inhibitors.

Most of the time corrosion occurs as a result of more than one mechanism either simultaneously or successively. Differential aeration cells can be created between normally highly oxygenated surface and metal under macro fouling or even under a thin layer of biofilm. The formation of various corrosion products on the metal slows down the corrosion process. But organisms disrupt these films and stimulate corrosion process. Hydrogen sulphide produced by sulphate-reducing bacteria under anaerobic conditions cause serious corrosion problems. There are several groups of bacteria, which are strongly associated with corrosion. Sulphate reducing bacteria, iron bacteria, slime forming bacteria, sulphur oxidizing bacteria and nitrogen utilizing bacteria are the important ones. Sulfate reducers of the genus Desulfovibrio are commonly reported groups.

PROTECTION METHODS AGAINST CORROSION

Prevention In the ocean where there are continuously changing physical, chemical and biological parameters it is often difficult to predict biological corrosion. Most often it is unexpected and is difficult to control, once established in the system. To prevent or control the problem, the following methods are used.

i. Use of biocides to control the biological activity.
ii. Use of anticorrosive coatings and application of Cathodic protection procedures
iii. Upgradation of material
iv. Use of physical barriers/wrappings

RED TIDES: CAUSATIVE FACTORES AND THE EFFECT OF THE ORGANSIMS ON THE MARINE ENVIRONMENT

Red tide is a colloquial term used to refer to one of a variety of natural phenomena known as *harmful algal blooms* or *HABs*. The term *red tide* specifically refers to blooms of a species of dinoflagellate known as *Karenia brevis*. It is sometimes used to refer more broadly to other types of algal blooms as well.

The term *red tide* is being phased out among researchers for the following reasons:

1. Red tides are not necessarily red and many have no discoloration at all.
2. They are unrelated to movements of the tides.

3. The term is imprecisely used to refer to a wide variety of algal species that are known as bloom-formers.

Red tide is a common name for a phenomenon known as an algal bloom (large concentrations of aquatic microorganisms) when it is caused by a few species of dinoflagellates and the bloom takes on a red or brown color. Red tides are events in which estuarine, marine, or fresh water algae accumulate rapidly in the water column, resulting in coloration of the surface water. It is usually found in coastal areas. It kills many manatees every year.

CAUSATIVE AGENT

These algae, known as phytoplankton, are single-celled protists, plant-like organisms that can form dense, visible patches near the water's surface. Certain species of phytoplankton, dinoflagellates, contain photosynthetic pigments that vary in color from green to brown to red.

When the algae are present in high concentrations, the water appears to be discolored or murky, varying in color from purple to almost pink, normally being red or green. Not all algal blooms are dense enough to cause water discoloration, and not all discolored waters associated with algal blooms are red. Additionally, red tides are not typically associated with tidal movement of water, hence the preference among scientists to use the term algal bloom.

Some red tides are associated with the production of natural toxins, depletion of dissolved oxygen or other harmful effects, and are generally described as harmful algal blooms. The most conspicuous effects of these kinds of red tides are the associated wildlife mortalities of marine and coastal species of fish, birds, marine mammals, and other organisms.

CAUSES

The occurrence of red tides in some locations appears to be entirely natural (algal blooms are a seasonal occurrence resulting from coastal upwelling, a natural result of the movement of certain ocean currents) while in others they appear to be a result of increased nutrient loading from human activities. The growth of marine phytoplankton is generally limited by the availability of nitrates and phosphates, which can be abundant in agricultural run-off as well as coastal upwelling zones. Coastal water pollution produced by humans and systematic increase in sea water temperature have also been implicated as contributing factors in red tides.

Other factors such as iron-rich dust influx from large desert areas such as the Saharan desert are thought to play a major role in causing red tides. Some algal blooms on the Pacific coast have also been linked to occurrences of large-scale climatic oscillations such as El Niño events. While red tides in the Gulf of Mexico have been occurring since the time of early explorers such as Cabeza de Vaca, it is unclear what initiates these blooms and how large a

role anthropogenic and natural factors play in their development. It is also debated whether the apparent increase in frequency and severity of algal blooms in various parts of the world is in fact a real increase or is due to increased observation effort and advances in species identification methods. In particular, the levels of nitrogen, phosphorous, and other nutrients in coastal waters are increasing due to runoff from fertilizers and animal waste. Complex global changes in climate also may be affecting red tides. Water used as ballast in ocean-going ships may be introducing dinoflagellates to new waters.

EFFECTS OF RED TIDE ON MARINE ENVIRONMENT

Sometimes the dinoflagellates involved with red tides synthesize toxic chemicals. Genera that are commonly associated with poisonous red tides are *Alexandrium*, *Dinophysis*, and *Ptychodiscus*. The algal poisons can accumulate in marine organisms that feed by filtering large volumes of water, for example, shellfish such as clams, oysters, and mussels. If these shellfish are collected while they are significantly contaminated by red-tide toxins, they can poison the human beings who eat them. Marine toxins can also affect local ecosystems by poisoning animals. Some toxins, such as that from *Ptychodiscus brevis*, the organism that causes Florida red tides, are airborne and can cause throat and nose irritations.

Red tides can cause ecological damage when the algal bloom collapses. Under some conditions, so much oxygen is consumed to support the decomposition of dead algal biomass that anoxic conditions develop. This can cause severe stress or mortality in a wide range of organisms that are intolerant of low-oxygen conditions. Some red-tide algae can also clog or irritate the gills of fish and can cause stress or mortality by this physical effect.

Saxitoxin is a natural but potent neurotoxin that is synthesized by certain species of marine dinoflagellates. Saxitoxin causes paralytic shellfish poisoning, a toxic syndrome that affects humans who consume contaminated shellfish. Other biochemicals synthesized by dinoflagellates are responsible for diarrhetic shellfish poisoning, another toxic syndrome. Some red tide dinoflagellates produce reactive forms of oxygen—superoxide, hydrogen peroxide, and hydroxyl radical—which may be responsible for toxic effects. A few other types of marine algae also produce toxic chemicals. Diatoms in the genus *Nitzchia* synthesize domoic acid, a chemical responsible for amnesic shellfish poisoning in humans.

Paralytic, diarrhetic, and amnesic shellfish poisoning all have the capability of making large numbers of people ill and can cause death in cases of extreme exposure or sensitivity. Because of the risks of poisoning associated with eating marine shellfish, many countries routinely monitor the toxicity of these foods using various sorts of assays. One commonly used bioassay involves the injection of laboratory mice with an extract of shellfish. If the mice develop diagnostic symptoms of poisoning, this is an indication of contamination of the shellfish by a marine toxin. However, the mouse bioassay is increasingly being replaced by more accurate methods of

determining the presence and concentration of marine toxins using analytical biochemistry. The analytical methods are generally more reliable and are much kinder to mice.

Marine animals can also be poisoned by toxic chemicals synthesized during blooms. For example, in 1991 a bloom in Monterey Bay, California, of the diatom *Nitzchia occidentalis* resulted in the accumulation of domoic acid in filter-feeding zooplankton. These small animals were eaten by small fish, which also accumulated the toxic chemical and then poisoned fish-eating cormorants and pelicans that died in large numbers. In addition, some humans who ate shellfish contaminated by domoic acid were made ill.

In another case, a 1988 bloom of the planktonic alga *Chrysochromulina polylepis* in the Baltic Sea caused extensive mortalities of various species of seaweeds, invertebrates, and fish. A bloom in 1991 of a closely related species of alga in Norwegian waters killed large numbers of salmon that were kept in aquaculture cages. In 1996, a red tide killed 149 endangered manatees (*Trichechus manatus latirostris*) in the coastal waters of Florida.

Even large whales can be poisoned by algal toxins. In 1985, 14 humpback whales (*Megaptera novaeangliae*) died in Cape Cod Bay, Massachusetts, during a five-week period. This unusual mortality was caused by the whales eating mackerel (*Scomber scombrus*) that were contaminated by saxitoxin synthesized during a dinoflagellate bloom. In one observed death, a whale was seen to be behaving in an apparently normal fashion, but only 90 minutes later it had died. The symptoms of the whale deaths were typical of the mammalian neurotoxicity that is associated with saxitoxin, and fish collected in the area had large concentrations of this very poisonous chemical in their bodies.

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